Assessing Forest Bird Exposure and Effects from Monosodium Methanearsonate (MSMA) during the Mountain Pine Beetle Epidemic in British Columbia

Christy Morrissey, Patti Dods, Courtney Albert, William Cullen, Vivian Lai, Tony Williams, John Elliot

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ABSTRACT

Recent and historical outbreaks of the Mountain Pine beetle (MPB) (*Dendroctonus ponderosae* Hopkins) have caused significant damage to forests in British Columbia through destruction of millions of hectares of pine forest. Management strategies have employed a variety of techniques to reduce timber losses from beetle outbreaks including the use of an arsenic-based pesticide monosodium methanearsonate (MSMA). We conducted a 4 year study in the Cascades (Merritt) Forest District to assess the threat of MSMA exposure to avian predators that feed directly on bark beetles, through a combination of field and laboratory methods. These include: arsenic analysis/arsenic speciation of bark beetles; scores of debarking on infested pine trees as an index of woodpecker foraging; blood and feather sampling of wild woodpeckers and other forest passerines to measure arsenic residues; focal observations of radio tagged woodpeckers to assess foraging behaviour and use of MSMA stands; forest bird surveys to assess species composition and abundance in MSMA stands relative to untreated areas; and insect (bark beetle) trapping to assess food supply and efficacy of MSMA in reducing MPB emergence.

We found concentrations of total arsenic in MPB from MSMA treated trees ranged from 1.3 – 700.2 µg/g dw (geometric mean 42.0 µg/g dw) with the organic metabolite monomethyl arsonic acid (MMAA) primarily comprising the total arsenic extracted. MPBs from reference trees had significantly lower total arsenic concentrations that averaged 0.19 µg/g dw. A negative relationship existed between the levels of debarking (foraging) on MSMA treated pine trees and the total arsenic in MPBs collected from those trees. The results suggest that woodpeckers were feeding more on non treated trees and those with poorer MSMA translocation and therefore, greater live beetle broods. Blood samples from three species of woodpeckers contained elevated levels of total arsenic but with large variability among individuals (geometric mean = 0.16 µg/g dw, range 0.05-2.14). Other forest birds including Mountain chickadee (*Parus gambeli*) had similar blood arsenic concentrations (geometric mean = 0.21 dw, range 0.02-2.20), while a pair of Red-breasted nuthatches (*Sitta canadensis*) averaged a low concentration (0.06 µg/g dw). Seventy-nine percent (42/53) of the individual blood samples were above the 0.07 µg/g level, which was identified as the mean reference value for blood arsenic in control birds from a MMAA dosing study of adult Zebra finches (*Taeniopygia guttata*) (Albert 2006).

Debarking indices indicated woodpecker foraging of MSMA treated trees was significantly lower than non treated trees. However, approximately 40% of MSMA trees had some evidence of woodpecker foraging (5-100% debarked). Focal observations of radio tagged

woodpeckers and point count surveys in MSMA treatment areas further confirmed several species of woodpeckers regularly used MSMA stands during the breeding season. Radio-tagged Hairy (*Picoides villosus*) and Three-toed (*Picoides* dorsalis) woodpeckers spent on average 13 and 23% (range 0-66%) of their time respectively in MSMA stands despite the fact that MSMA stands only comprised on average 1 to 2% of their core home range habitat (1 km^2). MSMA strongly reduced the emergence of several bark beetle (Coleoptera, Scolytidae) species including the MPB and there was a highly significant positive relationship between bark beetle (*Dendroctonus spp.*) abundance and Three-toed woodpecker abundance. The Three-toed woodpecker was the only bird species whose presence and abundance was significantly lower in MSMA treatment areas, which was attributed at least in part to a local reduction in food supply.

Given the scale of MPB infestations and the extent of MSMA use in British Columbia forests, this study addresses important knowledge gaps on the movement of MSMA from treated trees to bark beetles and avian predators and the potential negative impact that bark beetle management practices using pesticides can have on woodpecker populations that rely on the beetles and their host trees.

RÉSUMÉ

Les infestations récentes et passées du dendroctone du pin ponderosa (DPP) (*Dendroctonus ponderosae* Hopkins) ont causé de lourds dégâts dans les forêts de la Colombie-Britannique et ont entraîné la destruction de millions d'hectares de forêt de pins. Les stratégies de lutte ont fait appel à diverses techniques pour réduire les pertes de bois causées par les infestations de ce ravageur, y compris au méthylhydrogénoarsonate de sodium (MSMA), un pesticide à base d'arsenic. Nous avons utilisé une combinaison de méthodes de terrain et de laboratoire pour effectuer une étude de quatre ans dans le district forestier Cascades (Merritt) afin d'évaluer le risque d'exposition au MSMA des oiseaux prédateurs qui se nourrissent directement de scolytes. Ces méthodes étaient notamment les suivantes : analyse/caractérisation des espèces d'arsenic chez les scolytes; notation (en pourcentage) de l'écorçage de pins infestés pour établir un indice d'alimentation des pics; prélèvement d'échantillons de sang et de plumes chez des pics sauvages et autres passereaux forestiers pour mesurer les résidus d'arsenic; observations localisées de pics munis de radioémetteurs pour évaluer le comportement de recherche de nourriture et l'utilisation des peuplements traités au MSMA; recensements des oiseaux forestiers pour effectuer une évaluation comparative de la composition et de l'abondance des espèces dans les peuplements traités et non traités au MSMA; et piégeage d'insectes (scolytes) pour évaluer l'approvisionnement en nourriture et le degré de réduction de l'émergence du DPP procuré par le MSMA.

D'après les résultats de nos analyses, les concentrations d'arsenic total chez les spécimens de DPP provenant d'arbres traités au MSMA variaient de 1,3 à 700,2 µg/g p.s. (moyenne géométrique de 42,0 µg/g p.s.), et l'acide méthylarsonique (MMAA) était le métabolite organique constituant l'essentiel de l'arsenic total extrait. Les spécimens de DPP provenant d'arbres témoins avaient des concentrations beaucoup plus faibles d'arsenic total que la moyenne de 0,19 µg/g p.s. Il existait une corrélation négative entre les degrés d'écorçage (recherche de nourriture) des pins traités au MSMA et l'arsenic total chez les spécimens de DPP récoltés dans ces arbres. Les résultats obtenus laissent supposer que les pics s'alimentaient davantage sur les arbres non traités et sur les arbres dans lesquels le MSMA circulait moins bien et qui, par conséquent, abritaient un couvain plus abondant. Les échantillons sanguins prélevés chez trois espèces de pics présentaient des concentrations élevées d'arsenic total mais une grande variabilité d'un individu à l'autre (moyenne géométrique = 0,16 µg/g p.s., plage de 0,05 à 2,14). D'autres oiseaux forestiers, y compris la Mésange de Gambel (*Parus gambeli*), avaient des concentrations sanguines d'arsenic

similaires (moyenne géométrique = 0,21 p.s., plage de 0,02 à 2,20), alors qu'un couple de Sittelles à poitrine rousse (*Sitta canadensis*) avait, en moyenne, une faible concentration (0,06 µg/g p.s.). Soixante-dix-neuf pour cent (42/53) des échantillons sanguins individuels présentaient une concentration supérieure à 0,07 µg/g, une concentration établie comme étant la valeur de référence moyenne de l'arsenic dans le sang chez des oiseaux témoins lors d'une étude de dosage du MMAA chez des Diamants mandarins adultes (*Taeniopygia guttata*) (Albert, 2006).

D'après les indices d'écorçage, les pics s'alimentaient beaucoup moins dans les arbres traités au MSMA que dans les arbres non traités. Toutefois, environ 40 % des arbres traités au MSMA portaient des traces d'alimentation de pics (écorçage de 5 à 100 %). Des observations localisées de pics munis de radioémetteurs et des dénombrements ponctuels dans les secteurs traités au MSMA sont venus confirmer que plusieurs espèces de pics utilisaient régulièrement les peuplements traités durant la période de reproduction. Les Pics chevelus (*Picoides villosus*) et les Pics à dos rayé (*Picoides dorsalis*) munis de radioémetteurs passaient respectivement une moyenne de 13 et de 23 % (plage de 0 à 66 %) de leur temps dans les peuplements traités au MSMA, même si les peuplements traités ne représentaient en moyenne que 1 à 2 % de l'habitat de leur domaine vital principal (1 km^2). Le MSMA a fortement réduit l'émergence de plusieurs espèces de scolytes (Coléoptères, Scolytidés), y compris le DPP, et une corrélation positive très significative s'observait entre l'abondance de scolytes (genre *Dendroctonus*) et celle du Pic à dos rayé. Ce dernier était la seule espèce d'oiseau dont la présence et l'abondance étaient beaucoup plus faibles dans les secteurs traités au MSMA, une situation qui a été attribuée, du moins en partie, à une réduction locale de la quantité de nourriture disponible.

Compte tenu de l'ampleur des infestations du DPP et du degré d'utilisation du MSMA dans les forêts de la Colombie-Britannique, la présente étude comble d'importantes lacunes dans nos connaissances sur le mouvement du MSMA entre les arbres traités et les scolytes et oiseaux prédateurs et aborde l'impact négatif potentiel que les pratiques de lutte contre les scolytes fondées sur l'emploi de pesticides peuvent avoir sur les populations de pics qui dépendent des scolytes et de leurs arbres hôtes.

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STUDY OBJECTIVES

The field study was designed to assess the following objectives with respect to the potential impact of MSMA to insectivorous forest birds, primarily woodpeckers, in the southern interior of B.C. where Mountain Pine Beetle is at epidemic levels and MSMA treatments have been increasingly used as a means of controlling further infestation:

- To assess the degree of MSMA availability to birds through chemical analysis of bark beetles;
- To determine the level of MSMA exposure to woodpeckers by evaluating foraging activity on MSMA trees and by collecting blood from wild insectivorous birds known to prey on MPB;
- To assess the efficacy of MSMA treatments in reducing bark beetle emergence; and
- To evaluate the overall impact of MSMA pesticides to forest birds by providing an exposure and hazard assessment.

INTRODUCTION

An extensive outbreak of Mountain Pine beetle (*Dendroctonus pondersae Hopkins*) (MPB) during the early part of the 21st century in British Columbia's interior forests has posed a serious challenge to the forest industry. The current outbreak of the MPB has been ongoing for approximately 10 years, but the current infestation has exponentially increased to become the largest insect epidemic in North American history (Canadian Forest Service 2005). Latest reports of the beetle epidemic estimate that over 7.1 million hectares are affected by the beetle (B.C. Ministry of Forests 2004). The cause of the epidemic has been attributed to a combination of factors including a series of unusual hot dry summers and mild winters in central B.C., ineffective control measures during the initial outbreak, fire suppression, and large monoculture stands of mature, susceptible Lodgepole pine (*Pinus contorta*), the provinces most commercially harvestable tree species (Canadian Forest Service 2005).

The chemical monosodium methanearsonate (MSMA) has been used for over 20 years by the British Columbia forest industry in attempt to suppress or contain local bark beetle infestations, specifically MPB and Spruce beetle (*Dendroctonus rufipennis*). It is an organic arsenic compound, applied in the United States as an herbicide and defoliant for cotton, non-bearing citrus and nut crops, golf courses, recreational areas and rights of way (U.S. Environmental Protection Agency 2006). In Canada, it has been registered for use in the forest industry primarily to combat bark beetle infestations and also for tree thinning. Although several strategies have been applied for suppressing incipient beetle populations, the pesticide MSMA has been favored by foresters under certain conditions for its low cost and ease of use in remote areas where harvesting is not feasible (Canadian Forest Service 2005).

Adult MPBs typically have a one-year life cycle attacking live Lodgepole pine trees usually in mid to late July, constructing galleries and laying eggs which mature into larvae that overwinter, pupate and emerge the following summer. Under normal conditions, MPBs play an important ecological role in forest ecosystems by attacking old or weakened trees allowing younger forests to develop while providing an important food source to predators such as woodpeckers. However, recent years have seen beetles attacking healthy pines in large numbers.

Application procedures for MSMA treatment of Lodgepole pine infested with MPB follow a series of discrete steps designed specifically to target the beetle and minimize the impact to surrounding environment. In some cases, stands are pre-baited with semiochemicals (pheromones) to attract and attempt to isolate the beetles to an area and minimize the damage

to susceptible trees. Pine trees are targeted for MSMA application while the beetle is in the egg or early larval stages within 3-4 weeks post attack. Due to differences in timing of beetle emergence and attack, some sites may be visited 2 or more times in attempt to treat all the infested pines in an area. An axe frill is cut at the base of the tree to penetrate the cambium and MSMA (Glowon®, United Agri Products Canada) (0.32 kg elemental As (as MSMA)/L water and additives) is applied with a squirt bottle into the frill at a rate of 1 ml of Glowon® per 2.5 cm of tree circumference (B.C. Ministry of Forests 1995). The pesticide translocates through the xylem into the phloem resulting in death of the tree and direct and indirect toxicity to the beetles (Maclauchlan *et al.* 1988a, Manville *et al.* 1988). Typically, 10-50 trees are treated per site and trees are not removed following treatment. It is estimated that approximately 640-960 kg of MSMA was used annually in B.C. (Dost 1995). In the Cascades (Merritt) Forest District alone, approximately 68,000 trees were treated with MSMA in a period of 5 years (2000-2004) (BC MoF/MWLAP unpublished data). Based on estimates of reported usage for bark beetle control during 1995-2004 inclusive (D. Cronin, MWLAP, *pers. comm.*), almost 500,000 trees have been treated with MSMA in all British Columbia.

Given the current MPB epidemic, the increased use of MSMA in many areas of British Columbia may lead to significant arsenic loadings to local ecosystems. On the basis of limited information on organic arsenic toxicity available at the time, Dost (1995) reported that "Existing evidence indicates that the use of MSMA in bark beetle control does not impose measurable risk on applicators, the public or wildlife". This conclusion was reached in part because the biomethylation of inorganic arsenic was generally considered a detoxification process. In mammals, dimethlylarsinic acid (DMAA) has low acute toxicity and is readily excreted (Marafante *et al.* 1987, Hughes and Kenyon 1998). However, current knowledge of MSMA metabolism and toxicity indicates that a series of oxidation and reduction reactions in metabolism of arsenic compounds can produce trivalent methylated forms, particularly $MMAA^{III}$ which are more toxic than even inorganic arsenite (Cullen and Reimer 1989, Styblo *et al.* 2000, National Research Council [NRC] 2001). In addition, the trivalent forms of monomethyl arsonic acid ($MMAA^{III}$) and dimethyl arsinic acid ($DMAA^{III}$) both have direct genotoxic action (Mass *et al.* 2001) and DMAA has carcinogenic properties (Wei *et al.* 1999). Therefore, methylation of arsenic is now recognized as a mechanism of toxicity rather than solely a detoxification process. As a result, MSMA is currently being re-evaluated for its future registration in both Canada and the United States (Pelley 2005).

Woodpeckers are frequently attracted to beetle outbreak areas due to increased food availability (Steeger and Dulisse 1997, Steeger *et al.* 1998, Fayt *et al.* 2005). Several species of

forest birds will alter their diet in response to beetle outbreaks. The diet of certain species such as the Three-toed woodpecker (*Picoides dorsalis*) and Black-backed woodpecker (*Picoides arcticus)* is reported to consist of 75-99% Scolytid beetle larvae by volume in areas of beetle outbreaks (Steeger *et al.* 1998). Other species such as chickadees and nuthatches also regularly feed on susceptible insects particularly during outbreaks (Steeger *et al.* 1998, K. Martin, UBC, *pers. comm.*). Given that insectivorous birds are frequently attracted to bark beetle outbreaks in forests due to increased food availability, we hypothesized that woodpeckers and other forest birds may be subsequently exposed to elevated concentrations of arsenic through contaminated invertebrate prey from MSMA treated trees.

A few studies have examined arsenic residues in MSMA treated trees (Maclauchlan *et al.* 1988a,b) and arsenic movement into surrounding vegetation (Norris *et al.* 1983). One study measured arsenic in beetles from treated trees, but used only one quarter strength MSMA and only analyzed burdens in adult beetles (Maclauchlan *et al.* 1988a). No studies have examined arsenic concentrations in beetle larvae that may survive in the treated trees for up to 1 year before emergence. Furthermore, those studies only measured total arsenic, not specific forms which may be toxicologically more relevant. While the toxicity of MSMA has been identified in several laboratory studies of select mammals and aquatic species (summarized by Dost 1995), there have been no studies conducted on wild insectivorous birds that potentially have direct exposure to MSMA.

From an environmental perspective, the movement of MSMA or its arsenic derivatives to non-target wildlife is of primary concern, but was assumed negligible given the discrete nature of the application procedure. Prior to this study, no formal investigations were conducted, and general observations by foresters and biologists did not identify exposure risks or toxicological concerns to wildlife (Dost 1995). Movement or exposure of MSMA to non-target wildlife would require 1) beetle accumulation of MSMA and/or MSMA metabolites; 2) beetle survivorship of MSMA treatment; 3) wildlife occupancy of treated stands and 4) wildlife consumption of contaminated beetles from MSMA treated trees. Therefore, our main objectives in this study were to assess the availability, exposure, and effects of MSMA to insectivorous forest birds, including woodpeckers and other cavity nesting birds, in the southern interior of B.C. where MPB infestations are at epidemic levels and MSMA treatments have been frequently used as a means of controlling further infestation.

CHAPTER 1:

ARSENIC RESIDUES IN BARK BEETLES FROM MSMA TREATED TREES

METHODS

Study Area

The study area was in the Cascades Forest District (formerly Merritt Forest District) with sites located primarily between Merritt and Princeton, British Columbia, Canada. We targeted forest areas which received recent MSMA treatments (i.e. treated within the previous year) using information provided by the B.C. Ministry of Forests and local timber harvesting operators (Weyerhauser, Tolko, Aspen, B.C. Timber Supply). Selected MSMA stands had approximately 50 to >200 recently treated trees at the site.

Bark Beetle Collections

We assessed the potential risk to avian predators through arsenic analysis of adult and larval/pupal MPB and other insects. Other insects sampled included predators of MPB (*Clerid* larvae), pine engravers (*Ips* adults), wood-boring beetles and unidentified insects. Insects were collected from infested Lodgepole pine trees treated with MSMA approximately 4 weeks (green attack) and almost 1 year (red attack) post treatment (June – August). We removed the bark with an axe and collected individuals from beetle galleries with forceps and transferred the insects into clean glass jars. Additional samples were collected from nearby Lodgepole pines that were also infested with MPB but not treated with MSMA (reference trees). In the spring and summer of 2002, 17 insect samples were collected from MSMA and reference trees that had evidence of woodpecker foraging activity (debarking index $\geq$ 3). In 2003, 2004 and 2005, we collected 23, 19 and 29 samples respectively of MPB in the same manner but from trees with different levels of debarking (debarking index 0-7). The debarking index refers to visual inspection of the whole tree to assess the amount of bark removal which directly represents the extent of woodpecker foraging, where 0 = no debarking, 1 = <5%, 2 = 5-10%, 3 = 11-20%, 4 = 21-40%, 5 = 41-60%, 6 = 61-80%, 7 = 81-100%.

Additional data were recorded at the time of beetle collections included the morbidity of the beetles, the life stage (adult or larval/pupal), the tree's diameter breast height (DBH), height of beetle collection in 2m intervals, and an index (0-7) for the amount of debarking on the tree by woodpeckers. The majority of samples (>90%) were collected within 2m above the axe frill where MSMA is applied. Each sample represented a composite of >10 individual beetles from a

single tree. A few samples (n = 3) with insufficient mass/beetles for arsenic analysis were pooled with another similar sample collected at the same site/time and with similar characteristics (i.e. same debarking score, life stage).

Arsenic Analysis

All beetle samples were analyzed for total arsenic. Bark beetles collected in 2002 and 2003 and a sub-sample in 2005 were further analyzed for organic and inorganic arsenic speciation. Sample preparation and analyses were performed at the laboratory of Dr. William Cullen at the Department of Chemistry, University of British Columbia.

Samples for total arsenic analysis were freeze dried (0.02-0.2 g) and acid digested using nitric acid and hydrogen peroxide. Samples after digestion were diluted appropriately with the rhodium-nitric acid solution and stored at 4°C until analysis. Total arsenic analysis was performed using inductively coupled plasma mass spectroscopy (ICP-MS). Signals were corrected according to the signal of the internal rhodium standard. Quality assurance of total arsenic analysis was maintained by analyses of procedural blanks and at least 2 certified reference materials (Dorm-2, Dolt-2 (National Research Council, Canada) and IAEA Fucus (National Institute of Standards and Technology, U.S. Department of Commerce) as well as an in house standard (Kelp powder). Results were typically within 5% of certified values (range 96-113 %). Reliable quantification of total arsenic in all samples (method detection limit) was typically between 0.02-0.03 µg/g.

Samples for speciation of arsenic were also freeze-dried (0.02-0.2 g dry weight) and extracted using a procedure similar to that previously described (Shibata and Morita 1992, Lai *et al.* 1997). Extracts were stored at -20□C and transferred to the cold room (~ 4□C) on the day of analysis. Speciation of extracts was performed using High Performance Liquid Chromatography (HPLC) combined with an ICP-MS detector. Arsenic compounds in the samples were identified and quantified by matching the retention times of the peaks in the chromatograms with those of known standards. Speciation consisted of identifying inorganic arsenic (arsenate, arsenite) and major organic forms monomethyl arsonic acid (MMAA), which is the unionized form of MSMA and dimethyl arsinic acid (DMAA). It is important to note that the analytical procedure is unable to detect the different valence states of the organic arsenicals (i.e. $MMAA^{V}$ and the more toxic form $MMAA^{III}$) and so we generically report MMAA and DMAA to jointly represent the pentavalent and trivalent forms.

Data Analysis

All of the total arsenic concentrations in bark beetles were log-normally distributed and subsequently transformed prior to performing statistical analyses. The data are reported as back-transformed geometric means and ranges where appropriate. Comparisons among beetle samples for effect of treatment, timing of collection, life stages, and species were initially analyzed using a series of one way analysis of variances (ANOVA) or two-sample t-tests. The significance level (0.05) was Bonferroni corrected (0.01) to adjust for multiple comparisons on the data set. We used a two-way ANOVA to examine the effects of treatment with MPB life stage (adult or larval/pupal), and treatment with species (MPB or other insects). We then used a multi-factor regression model to test for combined effects of treatment (MSMA or reference), year, morbidity of beetles, and species (MPB or other insects) to identify which variables were most important in predicting total arsenic concentrations. A Pearson correlation analysis identified the relationship between debarking scores of MSMA treated trees approximately 1 year after treatment and the associated concentration of arsenic in MPBs from those trees. We excluded non-MPB (other insects) and one extreme MPB outlier (700 ppm with debarking score of 5) from the Pearson correlation analysis.

RESULTS

Total Arsenic Residues in Bark Beetles

In total, 90 bark beetle samples were collected over 4 years and analyzed for total arsenic residues. Over half (54%) of the bark beetle samples from MSMA trees had arsenic residues < 50 ppm, while another 39% were in the 100-250 ppm range (Figure 1.1). Concentrations of total arsenic in adult and larval MPBs from MSMA treated trees ranged from 1.3 – 700.2 µg/g dw (geometric mean 42.0 µg/g dw) (Table 1.1). No differences existed in arsenic concentrations between sampling periods at 4 weeks (green attack) and 1 year (red attack) post treatment ($t_{65} = 0.56$, $p = 0.57$). MPBs from nearby reference trees had marginal or non-detectable arsenic concentrations (geometric mean 0.19 µg/g dw, range ND - 1.96 µg/g dw) that were significantly lower than MPBs from MSMA trees ($t_{76} = 14.2$, $p < 0.0001$). Adult MPB from MSMA trees were significantly higher in total arsenic than larval/pupal stages ($F_{2,75} = 128.2$, $p = 0.0002$) (Figure 1.2). Concentrations of total arsenic in other insects (non MPB) from MSMA treated trees ranged from 0.22 – 62.9 µg/g dw and were significantly lower than adult and larval MPB arsenic residues ($F_{2,86} = 93.8$, $p = 0.02$). MSMA treatment, treatment year, and beetle morbidity were all significant effects in the regression model for predicting total arsenic residues, however the beetle species (MPB or other insects) was not (whole model: $F_{7,81} = 36.2$,

Table 1.1: Summary of total arsenic measured in 90 composite samples of bark beetles (Mountain Pine Beetle (MPB), *Ips*, Cleridae and other insects) from MSMA treated and reference trees in the Cascades Forest District, B.C. from 2002-2005.

Treatment	Age/Spp	n	Morbidity	Geometric Mean Total [As] (µg/g)	Range Total [As] (µg/g)
MSMA	Adult MPB	15	dead	155.50	57.3-354.1
MSMA	Adult MPB	6	live	43.22	7.09-140.3
MSMA	Larval/Pupal MPB	5	dead	93.14	9.1-700.2
MSMA	Larval/Pupal MPB	32	live	20.00	1.3-327.4
MSMA	Ips	4	live	5.92	0.63-19.6
MSMA	Other insects	5	live	10.15	0.22-62.9
Reference	Adult MPB	6	dead	0.11	ND-1.06
Reference	Adult MPB	4	live	0.75	0.32-1.06
Reference	Larval/Pupal MPB	11	live	0.15	0.04-0.79
Reference	Cleridae	2	live	0.36	0.08-1.62

$p < 0.0001$). After accounting for combined effects within the model, the MSMA samples were still strongly elevated above reference samples ($p < 0.0001$); the 2002 treatment year had the highest arsenic concentrations but only significantly higher than 2001 ($p = 0.02$); and dead beetle samples contained significantly higher arsenic concentrations than live samples ($p = 0.0008$). Of particular note, a composite sample of adult MPB found alive in the summer following treatment contained arsenic concentrations up to 140 µg/g dw, while larvae were able to survive concentrations up to 327 µg/g dw.

We found a significant negative correlation ($r = -0.32$, $p = 0.018$) between mean total arsenic concentrations in bark beetles from MSMA trees and the amount of woodpecker debarking on those trees almost 1 year post treatment (Figure 1.3). This suggested that

woodpeckers were likely feeding more on trees with poor MSMA translocation, lower arsenic levels, and possibly larger live beetle broods.

Arsenic Speciation in Bark Beetles

The organic metabolite monomethyl arsonic acid (MMAA), which is the major form of MSMA at gastrointestinal pH, on average contributed 90-97 % to the total arsenic extracted from beetle samples in MSMA trees (Figure 1.4). We detected only trace amounts of inorganic arsenic in MPB samples from MSMA treated trees and dimethyl arsinic acid (DMAA) typically represented less than 1% of the total arsenic extracted. Although insect samples from reference trees had low or non-detectable arsenic residues, we found the arsenic species was primarily inorganic. Some reference adult MPB collected from green attack trees likely had been reared in an MSMA tree and carried residual arsenic contamination when they emerged and attacked an untreated tree. For example, the highest reference sample (1.96 µg/g) consisted of adult MPBs collected from a newly attacked tree (adult beetle galleries 4-10 cm) standing next to previously treated MSMA trees. Unlike most reference samples in which the arsenic was primarily inorganic, we further noted 2 adult MPB samples also taken from green attack trees neighboring MSMA, consisted entirely of MMAA.

DISCUSSION

Efficacy studies of organoarsenical herbicides, including cacodylic acid (DMAA) and MSMA at the current application rates or lower have demonstrated significant mortality and brood reduction in several bark beetle species (Chansler and Pierce 1966, Frye and Wygant 1971, Newton and Holt 1971, Buffam *et al.* 1973, Coulson *et al.* 1975, Manville *et al.* 1988, Maclauchlan *et al.* 1988a). Many of those studies attributed beetle mortality to a combination of factors including desiccation and fungal invasions in the tree which created conditions that inhibit bark beetle development. However, Manville *et al.* (1988) showed that MSMA had direct insecticidal properties on Douglas fir beetles (*Dendroctonus pseudotsugae* Hopkins). Most studies which measure arsenic after MSMA treatment focus on arsenic concentrations in tree phloem, sapwood and foliage with limited data available on residues in bark beetles. We found a wide range in arsenic residues (1.3 -700 ppm) in MPBs from MSMA treated trees. In comparison, one study that used ¼ strength MSMA reported similar or lower levels in adult MPB from 37-130 ppm (Maclauchlan *et al.* 1988a).

Bark beetle mortality from MSMA exposure reportedly varies and appears to be dependent on the media conditions. Maclauchlan *et al.* (1988a) found that arsenic concentrations in beetles varied in proportion to the amount in phloem ($r^2 = 0.84$), with beetles

accumulating approximately 2 times the amount of arsenic detected in phloem. Laboratory studies which examined the toxicity of MSMA, estimate complete mortality of MPB to occur at concentrations in media > 500 ppm (LC_{50} = 102 ppm) (Maclauchlan *et al.* 1988a) or 50 ppm (Manville *et al.* 1988). Manville *et al.* (1988) found no emergence of Douglas fir beetle progeny with phloem concentrations of 99 µg MMAA/g wet weight. The high concentrations and large degree of variability in our MPB samples, collected both alive and dead, suggests that variation in application rates, procedures and/or translocation of MSMA may be occurring in our study area which could be contributing to the variation in beetle concentrations and beetle survival. In addition, some beetles may be resistant to MSMA treatment and require much higher doses to cause mortality.

The timing of treatment appears a critical factor in affecting the survival of beetle progeny. Newton and Holt (1971) advise applying MSMA before beetle galleries and blue stain fungi are sufficiently advanced to prevent natural translocation of the pesticide. In addition, larvae that are exposed after they reach the third or fourth instar stage can reportedly survive higher concentrations of MSMA (>1200 ppm in phloem) (Machlauchlan *et al.* 1988a). We found evidence that some of the MPB emergence and attack occurred considerably earlier in the season during our study years. Window flight traps set up in 6 MPB infested stands (MSMA and reference) in the Merritt Forest District in 2005, showed a peak in MPB emergence occurred in the last week of May. This early emergence/attack would allow greater development of the larvae prior to MSMA treatment that typically takes place in August (see Chapter 3). This could explain why many of the bark beetles we collected were able to survive much higher concentrations (up to 327 µg/g dw in larvae) than previously measured. Consistent with other studies, we also observed high mortality of MPB from MSMA treated trees. However, extreme concentrations (up to 700 µg/g dw) were measured in some dead MPB samples collected in our study area. Assuming the beetles accumulated this arsenic prior to death, this concentration well exceeds what was anticipated for killing beetles and suggests the timing of treatment may be delayed and the more developed larvae were possibly resistant to MSMA.

Most previous studies examining the impact of MSMA to bark beetles only measured total arsenic, not specific forms which may be toxicologically more relevant. Arsenic is present is several chemical forms in the natural environment and its toxicity is dependent on the arsenic species and oxidation state (Cullen and Reimer 1989). Manville *et al.* (1988) found distributions of arsenic species in the phloem of treated and control trees that were very similar to that of our beetle samples. Phloem samples from MSMA trees contained primarily MMAA with only trace amounts of inorganic arsenic and DMAA, whereas control trees contained low arsenic residues

primarily in the form of inorganic arsenic. This suggests that the MMAA is not significantly changed when taken up by bark beetles from the phloem and is readily available to predators consuming the beetles. Until recently, organic arsenicals (e.g. MMAA and DMAA) were generally considered less toxic than inorganic arsenic compounds (arsenate and arsenite); however, studies in mammals have shown that trivalent methylated forms, particularly $MMAA^{III}$ are more toxic than even inorganic arsenite (NRC 2001, Styblo *et al.* 2000). Newton and Holt (1971) speculated that *in situ* biotransformation of the arsenicals produces by-products that are responsible for beetle mortality in MSMA treated trees. Arsenicals, both inorganic and organic, are now known to be readily transformed through a series of oxidation, reduction, methylation and demethylation reactions where intermediate metabolites can inherently cause direct toxicity to the organism (Cullen and Reimer 1989, Styblo *et al.* 2000). Therefore, the mortality of bark beetles in MSMA trees is likely a product of these biotransformation reactions.

It appears that woodpecker predation, as evidenced by the degree of debarking, was reduced on trees containing beetle samples with higher concentrations of arsenic. This suggests that although woodpeckers likely were not consuming the most contaminated beetles on a regular basis, they were still foraging on MSMA trees. Some of the treated trees were heavily debarked (up to 80%), but we do not know if those trees were fed on by one or by many woodpeckers; also we do not know the frequency of feeding. There are reports that woodpeckers frequently aggregate in beetle infested areas and densities can increase up to 30 fold in a relatively small area (Baldwin 1960, Koplin 1972), suggesting that heavy debarking of single trees is likely done by more than one individual.

Figure 1.1: Frequency distribution for total arsenic residues in bark beetle samples collected from MSMA treated trees in the Cascades Forest district 2002-2005. Each bar represents the proportion of all samples that were in each 50 ppm interval.

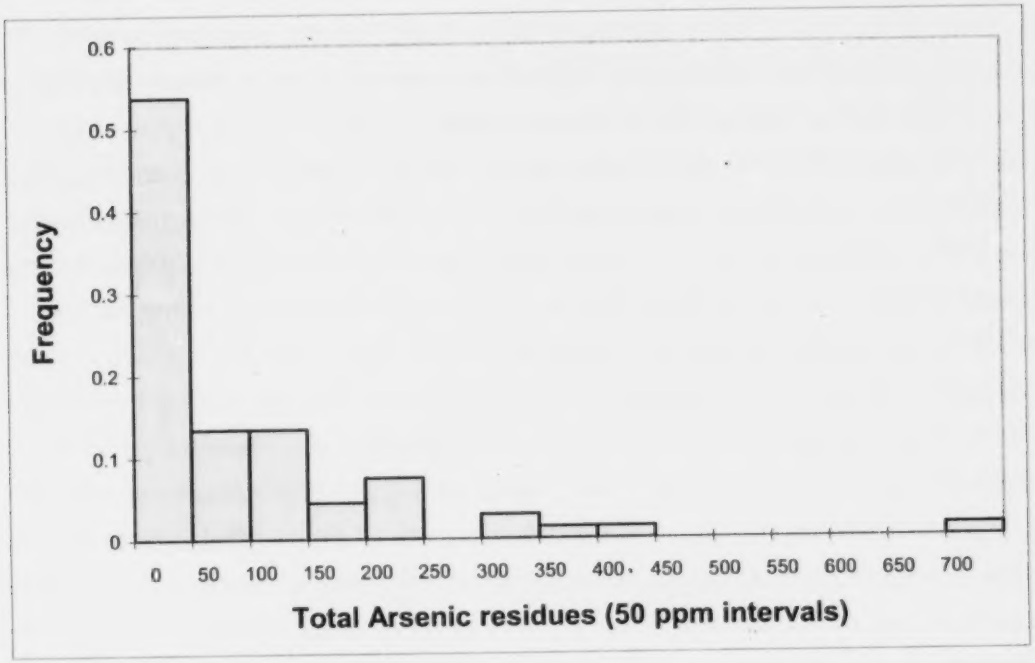


Figure 1.2: Geometric mean concentrations of total arsenic (µg/g) in all samples of adult and larval/pupal stages of Mountain Pine Beetle from MSMA and Reference trees collected in the Cascades Forest District, B.C., 2002-2005. Numbers above bars refer to sample sizes within each group.

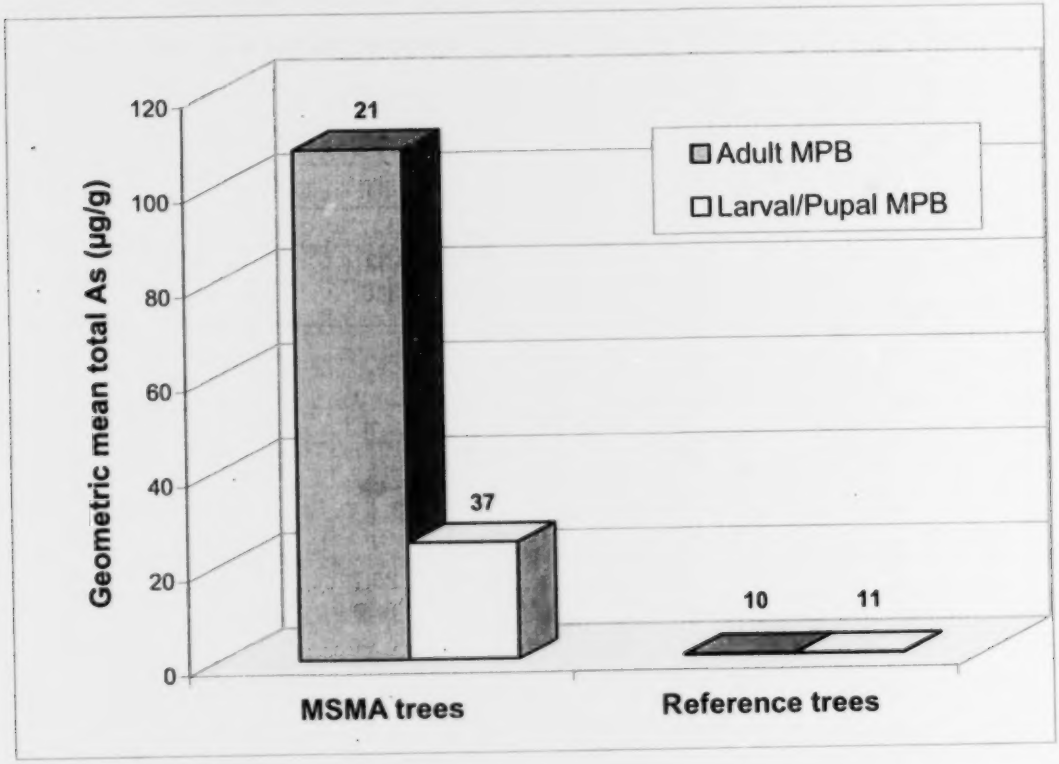


Figure 1.3: Relationship between debarking scores (amount of cumulative woodpecker foraging 10-12 months post treatment) and mean total arsenic concentrations of MPB collected from individual MSMA treated pine trees in the Cascades Forest District, B.C. See methods for description of debarking index. Values below points are the geometric mean arsenic concentrations (µg/g dw) and sample sizes. Error bars represent the standard error of the mean.

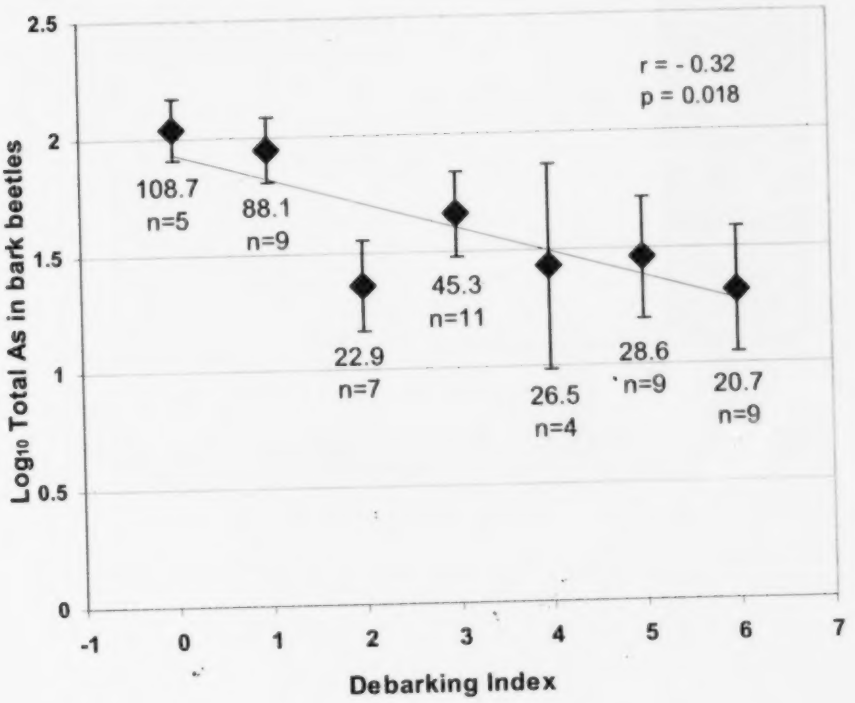
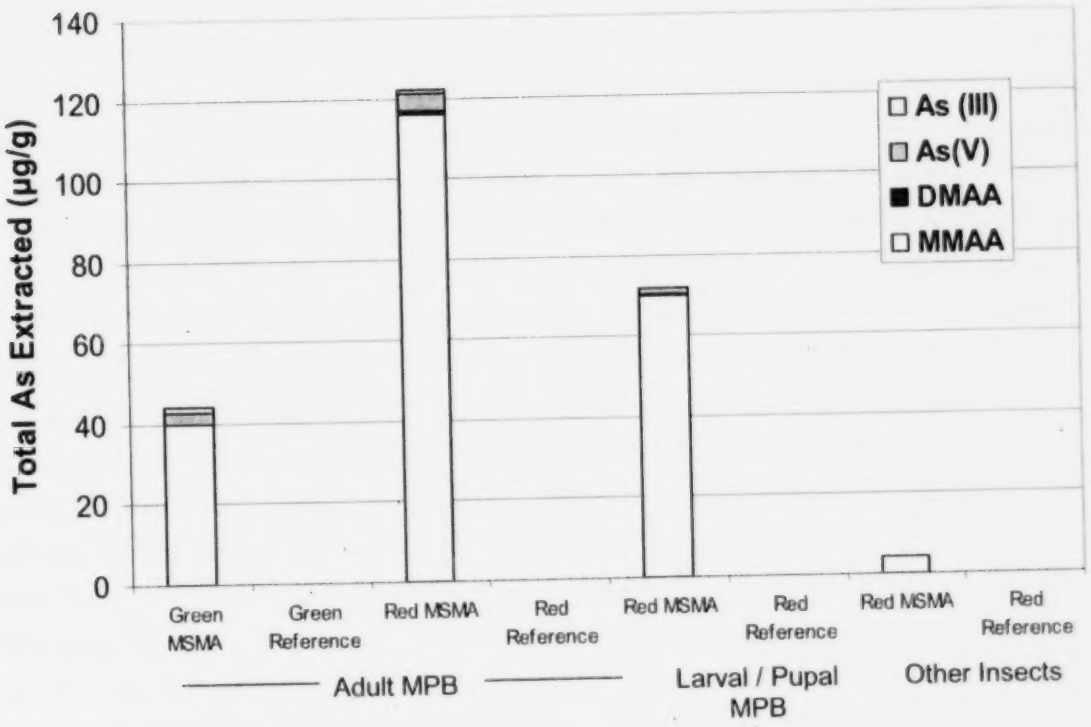


Figure 1.4: Arsenic speciation (As^{3+}, As^{5+}, DMAA, MMAA) in adult and larval/pupal stages of Mountain pine beetles (MPB) and other wood boring insects (other) collected from MSMA and reference trees in the Cascades Forest District, B.C., 2002-2005. Samples were collected 4 weeks (green attack) and approximately 1 year (red attack) following treatment.



CHAPTER 2:

AVIAN EXPOSURE TO MSMA: ARSENIC IN BLOOD AND FEATHERS

METHODS

Forest Bird Trapping

During the breeding seasons (April-July) of 2004 and 2005, Three-toed (TTWO) (*Picoides dorsalis*) and Hairy woodpeckers (HAWO) (*Picoides villosus*) occupying territories typically within 1 linear km of recent MSMA stands (treated in previous 2 years) were targeted for capture to obtain blood and feather samples. We concentrated on locating and capturing individuals or pairs of these species, as bark beetles are reported to comprise a large proportion of their diets (Steeger *et al.* 1998). In total, 23 birds (19 HAWO and 4 TTWO) were captured in mistnets using combinations of playback calls, dummy specimens and suet feeders as attractants or in bag nets placed over the entrance of the nesting cavity. An additional 9 Red-naped sapsuckers (RNSA) (*Sphyrapicus nuchalis*) were captured using the same techniques. Woodpeckers were aged by feather molt patterns (Pyle 1997), sexed, weighed, measured (tarsus, wing, tail, bill), banded, and 0.5 ml of blood taken by venipuncture of the jugular vein to use for total arsenic analysis. A sample of breast feathers was collected from woodpeckers for arsenic analysis. In 2005, two other species of forest birds breeding near MSMA stands were also captured at the nest for the purpose of taking blood for arsenic analysis, including 19 adult Mountain chickadees (*Parus gambeli*) and 2 adult Red-breasted nuthatches (*Sitta canadensis*). Chickadees and nuthatches were weighed, measured, banded and a 0.1 ml sample of blood was taken by venipuncture of the jugular vein. All procedures for trapping and handling birds were in accordance with Animal Care and Scientific Permits.

Arsenic Analysis

All bird blood and feather samples were analyzed for total arsenic. As with the bark beetles, sample preparation and analyses were performed at the laboratory of Dr. William Cullen at the Department of Chemistry, University of British Columbia using the same methods described in Chapter 1.

Data Analysis

All of the total arsenic concentrations in bird blood samples were log-normally distributed and subsequently transformed prior to performing statistical analyses. The data are reported as back-transformed geometric means and ranges where appropriate. Blood arsenic residues from

male and female breeding pairs (n = 12 pairs) across all species were significantly positively correlated (r = 0.64, p = 0.02). Therefore, in cases where we have data from both the male and female from the same nest, we used an average of the blood arsenic residues and treated the pair as a single sample for statistical analyses. Comparisons among bird species and sexes for total arsenic concentrations in blood were analyzed using a two-way ANOVA. Bird nest locations and recent MSMA treatments (2002-2004) were mapped using ArcMap®. We then summed the number of MSMA trees within 1 km^2 (564 m radius) of the nest site to examine correlations with blood arsenic residues.

RESULTS

Arsenic Residues in Bird Blood and Feathers

Blood samples collected from 5 species of cavity nesting forest birds in the Cascades Forest District in 2004 and 2005 revealed moderate but widespread exposure to MSMA (Figure 2.1). Birds were breeding typically within 1 linear km of a recent MSMA treatment area at the time of sampling. Blood arsenic concentrations in 3 species of woodpeckers ranged widely from 0.05-2.14 µg/g dw (geometric mean = 0.16 µg/g dw). Other forest birds occupying MSMA stands including adult Mountain chickadees (*Parus gambeli*) had similar blood arsenic concentrations (geometric mean = 0.21 µg/g dw, range 0.02-2.20), while a pair of Red-breasted nuthatches (*Sitta canadensis*) had low concentrations (0.06 and 0.07 µg/g dw). The highest blood arsenic residue (3.73 µg/g dw) was from a female Red-naped sapsucker caught in 2005 occupying an MSMA site that had 74 MSMA trees treated in the previous year. A weak negative correlation was observed between blood arsenic concentrations (all species) and the number of MSMA trees in a 1km^2 territory (r = -0.32, p = 0.04) (Figure 2.1). Given the large variability among individuals and the differences in sample sizes between the groups, we could not detect any patterns with respect to species or sexes ($F_{6,34}$ = 0. 43, p = 0.85).

Feathers collected from adult woodpeckers breeding near MSMA stands in 2004 similarly had variable total arsenic residues (Table 2.1). Age, sex and location did not reveal any patterns particularly with such a limited sample size. Feathers from woodpeckers were not analyzed in 2005.

Table 2.1: Summary of geometric mean total arsenic residues in breast feather samples of woodpeckers captured at the nest site in 2004 in the Cascades Forest District.

Species	n	Mean (µg/g dw)	Range (µg/g dw)
HAWO	8	0.70	0.19-3.95
TTWO	1	1.39	-
RNSA	2	0.20	0.13, 0.32
All birds	11	0.60	0.13-3.95

DISCUSSION

In general, there is limited published data for comparison of arsenic residues in blood of birds. Given the available evidence, blood arsenic concentrations in most woodpeckers and other forest passerines breeding near MSMA sites were elevated. Seventy-nine percent (42/53) of the individual blood samples were above 0.07 µg/g, which has been identified as the mean reference value for blood arsenic in control birds from a 2 week MMAA oral dosing study of adult Zebra finches (*Taeniopygia guttata*) (Albert 2006), and 51% (27/53) were greater then 0.14 (2 times higher than this reference value). The blood levels were within the range found in the low and medium dose groups consuming 8 and 24 µg MMAA/g bw/day (Albert 2006). Most blood samples were also above other reported reference concentrations; for example, Franklin's gulls (*Larus pipixcan*) sampled at an uncontaminated marsh site in the United States had 0.005-0.02 ppm (Burger and Gochfeld 1997). Total arsenic concentrations in blood of 11 species of birds sampled near a toxic spill from a mine site exhibited a range of concentrations, 0.006-0.19 µg/g dw, that were also generally less than most of our samples (Benito *et al.* 1999). Among 109 King (*Somateria spectablilis*) and Spectacled (*Somateria fischeri)* eider blood samples collected from a marine system in northern Alaska, only 2 birds had detectable concentrations of arsenic in blood at 0.55 and 0.80 µg/g dw (assuming 80% moisture) (Wilson *et al.* 2004). Since arsenic is a naturally occurring element with wide geographical differences in background concentrations, we would have ideally preferred to sample woodpeckers and other passerines in B.C. forests without MSMA application in order to obtain a representative reference value. However, we were logistically constrained from obtaining this data. Given that we were studying terrestrial birds, particularly passerines with direct oral exposure to the metabolite MMAA, we believe the concentrations of arsenic (0.07 µg/g) in control birds from the Zebra finch laboratory study are probably the best comparative value available at this time.

Although 79% of our blood samples were elevated above the reference value of 0.07 µg/g dw for Zebra finches dosed with MMAA, we found large variability in the levels of arsenic from woodpeckers and chickadees sampled in our study area. Blood arsenic residues did not associate significantly with species, despite data showing that Three-toed and Hairy woodpeckers feed heavily on MPB and other bark beetles during outbreaks (Otvos and Stark 1985, Villard and Beninger 1993, Villard 1994, Steeger *et al.* 1998, Fayt *et al.* 2005). This is in contrast to Red-naped sapsuckers which tend to feed more on sap and other insects, berries and spiders; or chickadees and nuthatches which also have a more varied diet of insects, seeds, nuts, berries and spiders (Steeger *et al.* 1998). The highest blood arsenic residue was detected in a female Red-naped sapsucker. This raises the question of whether another exposure route (i.e. ingestion of sap) is potentially equally or more important than the hypothesized main route of exposure from bark beetle consumption.

Most of the birds sampled were breeding in close proximity or even within MSMA stands; however, the amount of arsenic in blood was inversely related to the number of treated trees within 1 km^2 from the nest. We would expect that woodpecker species which consume bark beetles in large quantities (i.e. Three-toed and Hairy woodpeckers) would have blood arsenic residues which correlated positively to MSMA tree density. However, it is possible that birds nesting in close proximity to large MSMA stands feed farther away where MSMA has not reduced the food supply (see Chapter 4). Alternatively, this analysis, which produced only a weakly statistically significant correlation, may not be biologically significant because it includes only MSMA trees treated in 2002-2004, and does not account for the individual home range size of each species or bird.

Variation in toxico-kinetics of organic arsenicals, specifically rates of uptake and elimination, likely contributed to the variance among individual blood samples. Ingested methylated arsenicals typically have biological half-lives of only 30 hours (Klaassen 2001). Albert (2006) found that adult Zebra finches orally dosed with MMAA at 8, 24 and 72 µg/g rapidly accumulated arsenic in the blood and tissues in a dose dependent manner. After 14 days, the high dose group had significant total arsenic accumulation in blood, 3.8 µg/g, liver, 1.1 µg/g, kidney, 1.6 µg/g, brain, 3.7 µg/g, and carcass, 12.3 µg/g. However, based on a mass balance approach, finches also excreted over 90% of the arsenic detected. Pendleton *et al.* (1995) also found rapid accumulation of inorganic arsenic in mallard ducks (*Anas platyrhynchos)* from dietary exposure of 300 µg/g sodium arsenate. Mean arsenic concentrations in blood were 0.85 µg/g dw and equilibrium levels were typically around 1 µg/g, but arsenic was also rapidly lost following cessation of exposure (half life of 1 to 3 days).

Despite rapid elimination rates, highly soluble arsenic forms that are readily absorbed may present greater risk, at least for acute toxicity (Pendleton *et al.* 1995). MSMA is water soluble and readily absorbed by the gastrointestinal tract of mammals (Shariatpanahi and Anderson 1984, Jaghabir *et al.* 1988). Those studies also found rapid uptake and elimination curves for MSMA in sheep, goats and rabbits, which were similar to the results for mallards dosed with arsenate (Pendleton *et al.* 1995). The exposure of insectivorous birds foraging in MSMA treated stands will tend to be intermittent rather than continuous. Given the apparent rapid elimination of the chemical, we can infer that any findings of arsenic in blood significantly above reported background levels are strong evidence of recent feeding in MSMA treated stands. However, blood arsenic levels of wild birds only indicate recent exposure and are not appropriate for inferring quantitatively the amount of MSMA they are ingesting.

As with the blood samples, woodpecker feathers had variable total arsenic residues. Since we cannot be certain of where birds were foraging at the time of feather molt the previous year, feather arsenic residues in adult birds are not recommended as a reliable indicator of exposure to MSMA.

Figure 2.1: Relationship between the number of MSMA trees within 1 km^2 of the nest site and total arsenic (log_{10}) residues in blood of 5 species of forest birds breeding in the Cascades Forest District 2004-2005.

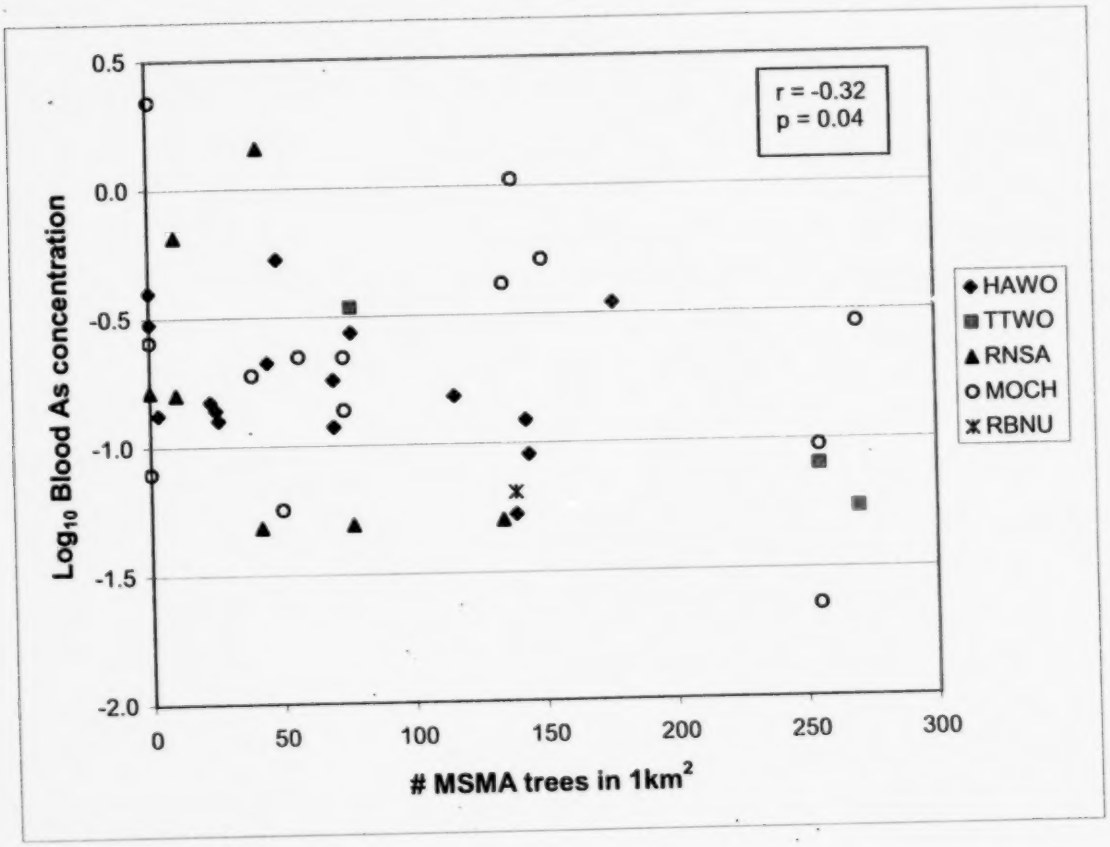
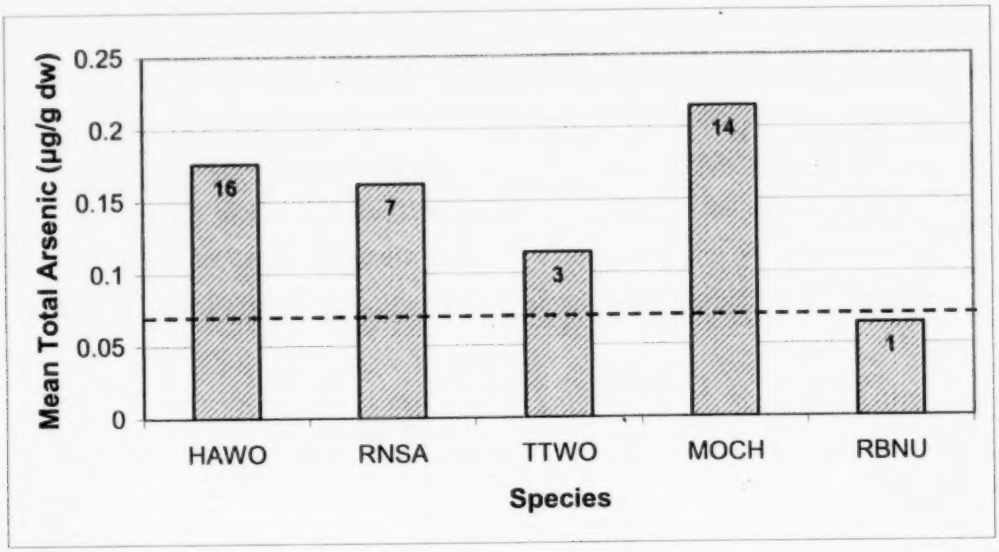


Figure 2.2: Geometric mean total arsenic concentrations in blood of 5 species of forest birds breeding within 1 linear km of MSMA treatment area in the Cascades Forest District, B.C. Numbers represent effective sample sizes for each species. The dashed line represents a mean reference value for unexposed birds based on dosing study by Albert (2006). (HAWO= Hairy Woodpecker, RNSA = Red-naped sapsucker, TTWO = Three-toed woodpecker, MOCH = Mountain chickadee, RBNU = Red-breasted nuthatch).



CHAPTER 3:

EFFICACY OF MSMA APPLICATIONS IN SELECTED MOUNTAIN PINE BEETLE INFESTED STANDS

METHODS

Insect Flight Traps

We designed a study to assess the efficacy of MSMA treatment and the composition and abundance of insects emergent in MPB infested stands particularly bark and ambrosia beetles (Coleoptera: Scolytidae), especially the genus *Dendroctonus*. A total of 62 window flight traps were placed in 3 stands treated with MSMA in August 2004 and in 3 reference stands infested with MPB in the same year but left untreated. Traps were constructed of two 30 cm square clear plastic panels that crossed at the centre, forming four 15 cm by 30 cm rectangles exposed in four directions (Safranyik *et al.* 2004). A funnel made from sheet metal attached below the panels and drained into a removable plastic collecting jar. The bottoms of the jars were removed and replaced with a fine mesh screen for drainage (Safranyik *et al.* 2004). Traps were set at a height of 1-2m and at a density of approximately 1 trap per 10 green attack trees at 30m intervals in a grid or as transects depending on the shape of the infestation at the site. All traps were set up between May 24 and 26, 2005 and taken down on September 7 and 8, 2005 for a total of 14 weeks. Captured insects were collected weekly until week 7, after which traps were checked on week 9 and week 14. Insects were stored in 70% ethanol for later identification. All insects were identified to order based on the descriptions and keys provided by Borror *et al.* (1989), Bright (1976) and Linton (2005). Insects of the order Coleoptera were further identified to family, and beetles of the Family Scolytidae were identified to species whenever possible.

Vegetation Sampling

We examined the tree composition at each site to account for its potential effects on insect numbers. Vegetation was sampled around 3-4 traps that were selected randomly at each of the 6 sites. All live and dead tree species with a diameter at breast height (DBH) $\geq$ 12.5 cm, the British Columbia Ministry of Forests inventory standard (Martin *et al.* 2004), were identified and counted in 15m radius around the traps. For Lodgepole pine, we recorded the stage of MPB attack and whether the tree had been treated with MSMA. Foliage colour and the presence of pitch tubes on the bole were used to assess the stage of MPB attack. Pitch tubes near the base of the tree and green to yellow foliage indicates the first year of attack and is classified as Green

attack (GA). Red attack (RA) refers to year two following initial attack and the foliage appears red. Grey attack (OA) represents trees more than two years after initial attack. Negron and Popp (2004) found that MPB mainly attacked ponderosa pine with a DBH $\geq$ 18 cm, so the $\geq$12.5 cm DBH standard is an acceptable criteria for this study.

Data analysis

The diversity of insects was examined by counting the number of orders, families in the order Coleoptera, and species in the family Scolytidae present at each site. Total insect abundance was measured as the total number of insects captured over the entire season. The standardized abundance was used for analysis to account for traps that were damaged or downed during visits and sites that had different numbers of traps. The abundance in each trap was standardized to 14 weeks and each site was standardized to 10 traps, a sampling effort of 140 trap weeks per site. The observed insect abundance was then multiplied by the trapping effort (140 trap weeks/actual number of trap weeks) to give the overall standardized abundance per site. Data was not normally distributed and variances were unequal so we log transformed the data prior to performing statistical analyses.

We compared the standardized abundance of Scolytidae and *Dendroctonus* at each site using an analysis of variance (ANOVA) followed by a Tukey HSD test. A non parametric Mann-Whitney U test was used to assess the difference in median site abundances of Scolytidae and *Dendroctonus* species between MSMA and reference stands. Variation in vegetation composition was also investigated by trap, site and treatment. Prior to analyses, tree abundance was converted into density (number/hectare). The site effects on tree composition were examined by performing an ANOVA followed by a Tukey HSD test on the densities per trap. We examined the differences in median tree densities between MSMA and reference areas using a Mann-Whitney U test on the mean site densities.

Correlation analyses were performed to examine the possible influence variation in vegetation composition might have on Scolytidae and *Dendroctonus* abundance. Pearson correlation analyses were performed between mean site densities of tree types and total site abundance of Scolytidae and *Dendroctonus*. Relationships were considered significant at $p \leq 0.05$. All tree species and types plus the 2 degree interaction terms were run through a stepwise regression as predictors of Scolytidae and *Dendroctonus* abundance. We repeated the Mann-Whitney U test to compare the median site abundances of Scolytidae and *Dendroctonus* by treatment using the predicted values from the models to account for any variation in vegetation composition at each site.

RESULTS AND DISCUSSION

Timing of emergence

Bark beetle emergence was followed over 14 weeks from late May to early September 2005. While the main peak of emergence occurred in late summer, we recorded an early spike in emergence in the first week the traps were out (May 24-31) (Figure 3.1). This has implications for bark beetle management because MSMA treatments are only effective when trees are treated within 3-4 weeks post attack before beetle galleries and blue stain fungi are sufficiently advanced to prevent natural translocation of the pesticide (Newton and Holt 1971). In addition, larvae that are exposed to MSMA after they reach the third or fourth instar stage of development can reportedly survive higher concentrations of MSMA (Machlauchlan *et al.* 1988). Trees in the Merritt Forest District were typically treated in August and September each year.

Composition and abundance of bark beetles in MSMA and reference stands

We captured a high diversity of bark beetle species from the Family Scolytidae, potential prey for woodpeckers (Table 3.1). There was no difference in diversity with respect to numbers of insect orders, Coleopteran families, or Scolytidae species between reference sites and MSMA sites. There were statistically significant differences in mean trap abundance of insects in the Scolytidae family and in the Genus *Dendroctonus* between MSMA and reference sites. Reference sites had higher median abundance of both Scolytidae (Figure 3.2a) and *Dendroctonus* (Figure 3.2b) ($U = 0.0$, $p < 0.05$ for both). Given that MSMA treatments were applied only to Lodgepole pine trees, it was not surprising that MSMA had the largest effect on reducing the abundance of MPB and also Lodgepole pine beetle (*Dendroctonus murrayanae*) (Figure 3.2b). MSMA treatments appeared relatively effective at reducing bark beetle emergence at the stand level (88% reduction in MPB and 80% reduction in Genus *Dendroctonus*). These results are consistent with efficacy studies of organoarsenical herbicides, including MSMA which have demonstrated high mortality and brood reduction in several bark beetle species (Chansler and Pierce 1966, Frye and Wygant 1971, Newton and Holt 1971, Buffam *et al.* 1973, Coulson *et al.* 1975, Manville *et al.* 1988, Maclauchlan *et al.* 1988a).

Some differences in vegetation composition between MSMA and reference sites were present (Table 3.2) including significant differences in the median density of fir, dead fir and dead Lodgepole pine between treatments ($U = 0.0$, $p < 0.5$ for all). Strong, positive correlations

Table 3.1: The standardized abundance of Scolytidae species encountered at each site in the Cascades Forest District. Totals are given for the commonly encountered Genus'. The table does not include unidentified individuals.

Species	MSMA sites			Reference sites		
	Goose	Thalia	Thynne	Swa 20	Swa 22	Swa 7
Crypturgus borealis	0	0	0	1.26	0	0
Polygraphus rufipennis	0	0	0	0	0	1.03
Conopthorous	0	0	0	0	0	1.03
Gnathotrichus sulcatus	0	1.44	0	0	0	0
Trypodendron	0	0	0	0	1.01	0
Hylastes nigrinus	0	0	0	1.26	0	0
Hylastes tenuis	0	0	0	1.26	2.01	0
Hylastinus obscurus	0	0	0	1.26	3.02	0
Procryphalus mucronatus	0	0	0	1.26	0	0
Pseudothysanaes rigidus	0	0	0	1.26	0	0
Pseudopityaphthorus pubipennis	0	0	0	1.26	0	0
Scolytus	3.11	0.72	0	0	0	0
total Dendroctonus	**13.48**	**10.77**	**7.00**	**42.88**	**76.55**	**37.06**
Dendroctonus ponderosae	8.30	3.59	1.00	26.49	55.40	24.71
Dendroctonus rufipennis	4.15	4.31	5.00	6.31	10.07	7.21
Dendroctonus valens	0	1.44	0	0	0	0
Dendroctonus murrayanae	1.04	0	1.00	8.83	5.04	5.15
Dendroctonus pseudotsugae	0	0.72	0	0	6.04	0
total Hylurgops	**7.26**	**15.79**	**10.00**	**31.53**	**41.29**	**10.29**
Hylurgops rugipennis	3.11	13.64	2.00	18.92	22.16	8.24
Hylurgops porosus	4.15	0	5.00	10.09	10.07	2.06
total Ips	**9.33**	**15.79**	**15.00**	**8.83**	**7.05**	**11.32**
Ips pini	2.07	7.18	2.00	1.26	1.01	2.06
Ips latidens	0	1.44	0	3.78	2.01	3.09
Ips montanus	0	0	1.00	1.26	0	0
Ips plastographus	1.04	2.15	2.00	0	0	0
Pseudips mexicanus	5.19	2.15	1.00	0	3.02	1.03
Ips emarginatus	0	0.72	0	0	0	0
total Dryocoetes	**2.07**	**1.44**	**6.00**	**6.31**	**2.01**	**6.18**
Dryocoetes affaber	2.07	0	5.00	6.31	2.01	3.09
Dryocoetes autographus	0	0	1.00	0	0	0
total Monarthrum	**1.04**	**0**	**0**	**1.26**	**0**	**2.06**
Monarthrum scutellare	0	0	0	0	0	2.06
Monarthrummali	1.04	0	0	0	0	0

Table 3.2: The mean tree type densities among MSMA sites (n=3) and reference sites (n=3) sampled near insect flight traps in 2005. Mean densities are given ± SE. The percent contribution of each tree type to the total vegetation composition for that treatment is shown in parentheses. Tree type densities that were significantly different ($p<0.05$) between MSMA and reference treatments are denoted by *.

Tree type	MSMA		Reference	
fir*	305.10 +/-68.98	(51.4)	14.15 +/-7.21	(1.6)
dead fir*	18.48 +/-13.76	(3.1)	0.00 +/-0.00	(0)
spruce	1.18 +/-1.18	(0.2)	164.74 +/-81.74	(18.8)
dead spruce	0.00 +/-0.00	(0)	3.15 +/-1.57	(0.4)
aspen	1.18 +/-1.18	(0.2)	1.57 +/-1.57	(0.2)
ponderosa pine	5.90 +/-4.25	(1.0)	0.00 +/-0.00	(0)
Lodgepole pine	243.38 +/-100.65	(41.0)	571.68 +/-241.47	(65.2)
dead Lodgepole pine*	18.87 +/-9.82	(3.2)	121.88 +/-13.64	(13.9)
GA	70.77 +/-40.68	(11.9)	103.80 +/-51.76	(11.8)
RA	1.18+/-4.23	(0.2)	7.86 +/-5.67	(0.9)
OA	9.44 +/-9.44	(1.6)	33.81 +/-2.84	(3.9)
TOTAL	**594.09 +/-172.28**		**888.18 +/-160.91**	

were observed between total abundance of Scolytidae and mean density of spruce and dead spruce at each site ($r = 0.95$, $p = 0.004$; $r = 0.97$, $p = 0.001$, respectively). Similar positive correlations also existed for *Dendroctonus* with spruce and dead spruce ($r = 0.87$, $p = 0.02$; $r = 0.83$, $p = 0.04$, respectively). Thus, some of the variability in overall emergence among individual sites could be attributed to the density of specific tree species that were infested with various bark beetles.

To control for the potential effect of vegetation differences among MSMA and reference stands, we applied a model predicting Scolytidae abundance constructed from a stepwise regression. Significant variables including the density of dead spruce, dead Lodgepole pine and red attack trees were incorporated as predictors. We found a significant difference in Scolytidae abundance between MSMA and reference treatments when using the predicted values from this model to correct for differences in vegetation among sites ($U=0.00$, $p < 0.05$). A similar model predicting *Dendroctonus* abundance incorporated density of spruce, Lodgepole pine, dead Lodgepole pine and significant interaction term (Lodgepole pine * dead Lodgepole pine) as the

predictors. Again, there was still a significant difference in *Dendroctonus* abundance between treatment types when using the predicted values for each site obtained from this model ($U=0.00$, $P<0.05$). Therefore, despite some random differences in vegetation composition between MSMA and reference stands, the effect of MSMA treatment was still strongly influencing the abundance of emergent bark beetles.

Our data presented here from 3 sites in the Merritt Forest District in 2005 demonstrate that at the stand level, MSMA treatments were reasonably effective. However, the Ministry of Forests has conducted routine monitoring of individual MSMA treated trees in the Merritt Forest District through random felling and bark removal to assess beetle mortality. Data from the previous year (2004) indicated MSMA killed on average 53% (range 10-100%) of individual beetle broods by tree (MoF unpublished data), which is considerably lower than our estimate of 88%. Low efficacy in 2004 was attributed to poor translocation of the pesticide because of dry conditions and possibly late timing of treatment when larval mining was too developed to allow translocation (Newton and Holt 1971, Maclauchlan *et al.* 1988a). In addition, we noted large variability among MSMA sites in numbers of green attack trees left untreated (Chapter 4) which would further reduce the efficacy of MSMA at the stand level. Thus, actual mean efficacy rates of MSMA treatment at the stand and tree level over multiple years and sites remains unclear.

Figure 3.1: Change in total number of Scolytidae bark beetles emergent and captured in insect flight traps set up in 6 MPB infested stands in the Cascades forest district. Traps were set up May 24-26, 2005 and collected weekly until week 7, after which the traps were collected on week 9 and 14. First 3 sites (Goose, Thalia, Thynne) are MSMA stands and last 3 sites (Swa20, Swa22, Swa7) are reference stands.

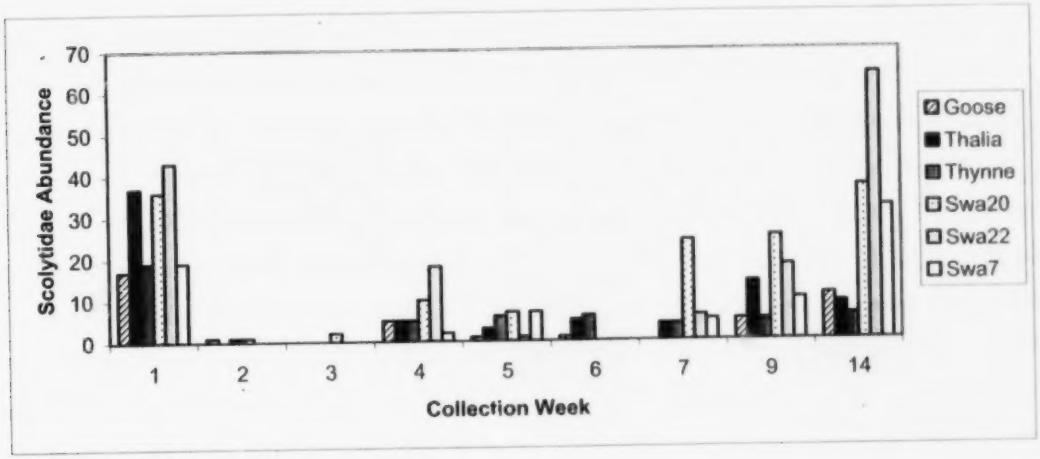


Figure 3.2a: Mean (± SE) standardized abundance of Scolytidae by genus captured in flight traps for MSMA (n = 6 sites) and Reference stands (n = 6 sites) of the Cascades Forest District, 2005.

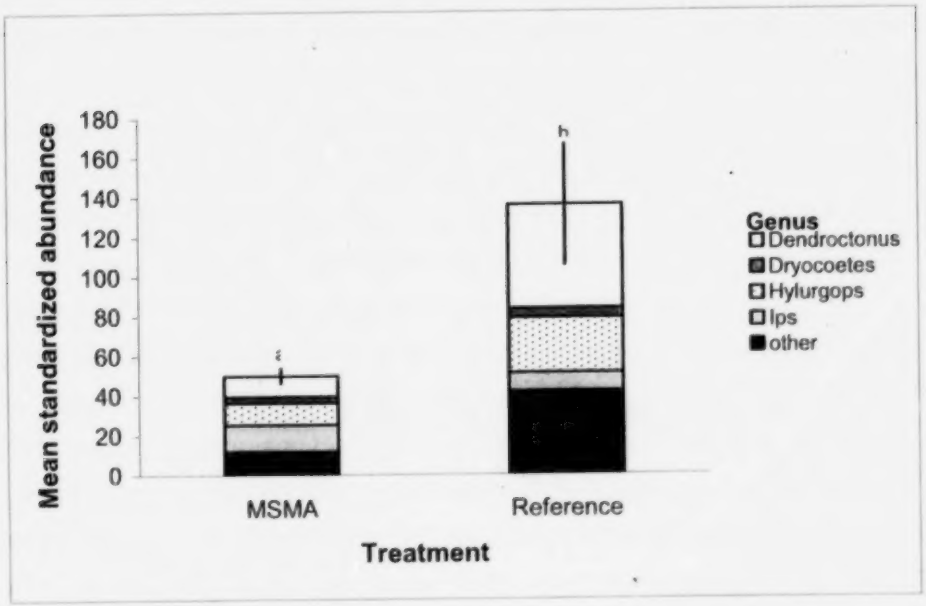
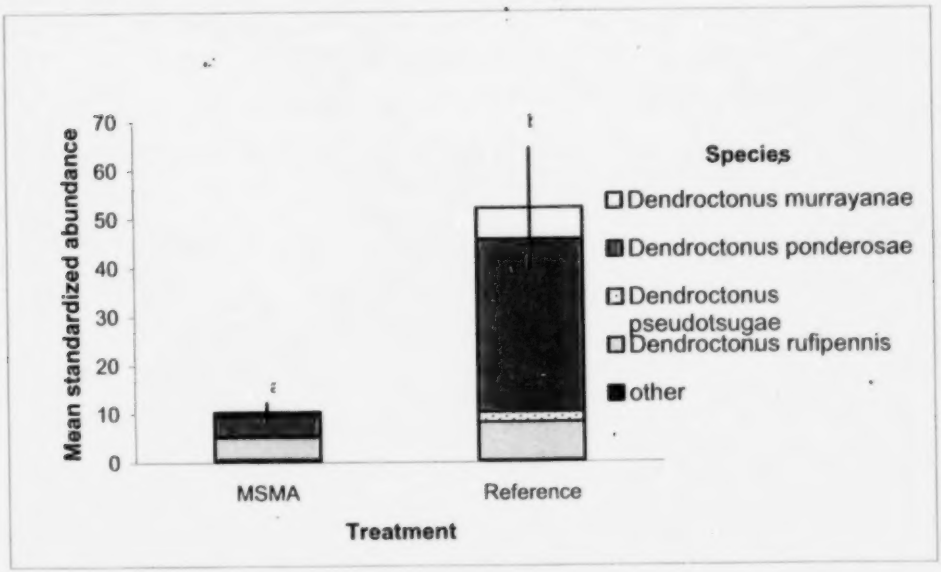


Figure 3.2b: Mean (± SE) standardized abundance of *Dendroctonus* by species captured in flight traps for MSMA (n = 6 sites) and Reference stands (n = 6 sites) of the Cascades Forest District, 2005.



CHAPTER 4:

WOODPECKER FORAGING BEHAVIOUR AND INTERACTIONS WITH MOUNTAIN PINE BEETLE: EFFECT OF PESTICIDE TREATMENT

METHODS

Woodpecker Debarking

In order to determine the intensity and timing of woodpecker use of MSMA treated trees, we recorded data on 150 MSMA-treated trees and 32 untreated infested trees (reference trees) over a time course during 2002-2003. Evidence of woodpecker foraging was scored by the amount of bark scaling or removal (debarking) at the time of treatment in July or August, approximately 3 months later in November, and again the following May or June. However, most reference trees could not be found the following spring/summer because they had been harvested. Over 3 years (2002, 2003 and 2005), 597 reference trees and 558 MSMA-treated trees were also assessed by single visits approximately one year after infestation/treatment (June-August). Reference trees were infested with MPB and located within MSMA treatment stands; therefore, factors that likely affect woodpecker foraging rates including forest composition, woodpecker density and bark beetle density should be the same for both MSMA and reference trees. Trees were scored by amount of debarking as an index of woodpecker foraging activity (index 0-7 where 0 = no debarking, 1 = <5%, 2 = 5-10%, 3 = 10-20%, 4 = 20-40%, 5 = 40-60%, 6 = 60-80%, 7= 80-100%), assessed for level and type of infestation, measured (DBH), and the height and type of foraging activity was recorded, as well as whether or not the stand was baited with insect pheromones.

Forest Bird Surveys

We conducted routine woodpecker surveys in spring 2005 at 8 MSMA sites and at 3 reference sites. We selected point count stations at MSMA sites by mapping the treated trees then placing a minimum of 4 stations at 200m intervals ensuring all stations were within 100m of the MSMA trees. One MSMA site was considerably smaller with only 2 playback stations and was thus, excluded from the analysis (report n = 7 MSMA sites). Reference site stations were also located 200m apart and encompassed the core area of the MPB infestation. The area surveyed ranged from approximately 12.6 to 25.1 hectares per site. Each site was surveyed every 3 days from late April to late May then every 2 weeks from June to July such that each site was surveyed at least 9 times over the course of the season. Playbacks of woodpecker

calls were conducted following published inventory methods for woodpeckers (Resources Inventory Committee 1999). Surveys were conducted by 2 observers from half an hour after sunrise until noon. Playback calls for Downy woodpecker (*Picoides pubescens*) (DOWO), Red-naped sapsucker (*Sphyrapicus nuchalis*) (RNSA), Three-toed woodpecker (*Picoides dorsalis*) (TTWO), Hairy woodpecker (*Picoides villosus*) (HAWO), Black-backed woodpecker (*Picoides arcticus*) (BBWO), Northern Flicker (*Colaptes auratus*) (NOFL) and Pileated woodpecker (*Dryocopus pileatus*) (PIWO) were played in that order. Each 20 second call was played twice, separated by a 30 second break, and calls of different species were separated by a two minute break. If an individual was heard from two stations it was recorded at the station it was closest to and a note was made that it was also heard at another station. When a woodpecker was heard but could not be identified (usually because it was pecking), a team member would search it out and identify it if possible. Other common forest bird species recorded during routine surveys included Mountain chickadee (*Poecile gambeli*) (MOCH), Black-capped chickadee (*Poecile atricapilla*) (BCCH), Chestnut-backed chickadee (*Poecile rufescens*) (CBCH) and Red-breasted nuthatch (*Sitta canadensis*) (RBNU).

As for the insect flight traps (Chapter 3), we examined the tree species composition at each of the 8 MSMA sites and 3 reference sites to account for its potential effects on woodpecker occupancy. Vegetation was sampled along four 100 meter (m) transects running north, south, east, and west from each of the playback stations at all sites. Observers walked the transect stopping every 10 m to count all live tree species (DBH >12.5cm) in a 10m half circle facing back to the station. For Lodgepole pine (*Pinus contorta*), we recorded the stage of MPB attack (GA = green attack (current year infestation), RA = red attack (previous year's infestation), OA = old attack (>2 year old infestation), the amount of debarking (index 0-7), and whether the tree had been treated with MSMA. The total area used for the vegetation surveys at each playback station was 6280 m^2. Given that most sites had 4 to 8 playback stations, we estimated the area surveyed for vegetation ranged from 2.5 to 5.1 hectares per site which was amounted to ~20% of the total bird survey area.

Woodpecker Radio-telemetry/Foraging Observations

During the breeding seasons of 2004 and 2005, TTWO and HAWO occupying territories within 1 km of recent MSMA stands (treated in previous 2 years) were targeted for capture to mount radio transmitters. We concentrated on capturing and locating nests of those species, as they are reported to consume a large number of bark beetles (Steeger *et al.* 1998). In total, 15 woodpeckers (12 HAWO and 3 TTWO) were captured on the nest with bag nets placed over the

entrance of the nesting cavity or in mistnets using combinations of playback calls, dummy specimens and suet feeders as attractants. Each bird had a 1.9 g radio transmitter (Holohil Ltd., Carp, Ontario, Canada) mounted to the central retrices with cyanoacrylate glue. Radios were expected to have a 14 week life span. The weight of the transmitter amounted to less than 3% of the lean body mass of the bird. Radio-tagged birds were monitored but data was not collected during the first week following transmitter attachment to allow time for acclimation. During the 2 year study, we successfully tracked 14/15 radio-tagged woodpeckers to obtain data on foraging behaviour during the breeding season. An additional 10 HAWO and 14 TTWO were opportunistically sighted during the course of the breeding season in 2005. We recorded details on the foraging substrate for these 24 woodpeckers (1 observation/day) at the time of first sighting to increase our sample size. However, only birds fitted with radio transmitters were used for analysis of MSMA stand use to avoid bias.

Each woodpecker was located within the forest by standard ground telemetry methods (Kenward 2001). We continued tracking the bird to record 20 minutes of visual observations with emphasis on foraging activities (2004) or one foraging observation until the bird flew out of sight (2005) at least once every 2 days (3x/week) for the duration of the breeding season or until transmitter failure. Foraging and home range data were collected by alternating observations between 3 different time intervals (7:00-10:00, 10:00-13:00 and 13:00-16:00). Each observation provided data on the bird's GPS location and details of the foraging substrate including tree species, level of infestation (GA, RA, OA), amount of debarking, height of foraging (by zones: bottom 1/3, middle 2/3, top 3/3), and whether it was occupying an MSMA treatment area.

Data Analysis

Debarking index scores are categorical values and average scores were analyzed to assess differences between treatments by year using non-parametric Wilcoxon rank sums. We tested which factors had the strongest influence on debarking scores using a mixed stepwise regression model which included the following variables: MSMA treatment, tree size (DBH), year of infestation, tree decay class (Resources Inventory Committee 1999), and the use of semiochemicals (pheromone baiting). Pearson correlations were used to determine if any relationships existed between average debarking scores at a site and variables including the density and number of MSMA trees, density and percentage of green attack trees, tree species composition, or abundance of TTWO. Significant correlations were used in an analysis of covariance (ANCOVA) to correct for their potential effect in determining the effect of MSMA on TTWO densities. Correlation analyses were also done to examine the potential effects of stand

composition on abundance of each bird species. Given the large number of correlations which could potentially yield random significant relationships, we used a Bonferroni correction to reduce the alpha level to 0.01.

The frequency of bird species detections represents the percentage of surveys in which each species was detected. Bird densities represent the mean number of birds counted per survey divided by the total area surveyed at a given site. Bird densities were compared between MSMA and reference sites using a two sample t-test and frequency of detections were analyzed for each species using a chi-square (χ^2) contingency table. Contingency tables were also used to assess differences among species and sexes in occupancy of MSMA stands and use of foraging substrates. Each day of foraging observations for a single bird contained data on multiple foraging substrates. We only used the first substrate (initial observation) that it was recorded on as this is considered the most statistically reliable for estimating proportional use (Bell *et al.* 1990). We used Pearson correlations to assess the effect of vegetation characteristics on bird density and TTWO density was regressed against the total number of bark beetles in the Genus *Dendroctonus* and Family Scolytidae as reported for the same sites in Chapter 3.

GIS Analysis of Woodpecker Radio-telemetry Data

Bird nest locations, all foraging locations, and recent MSMA treatments (2002-2004) were mapped using ArcMap® version 9 (ESRI, Redlands, California). Bird locations were identified by breeding phase (incubation, chick rearing, and post breeding). MSMA treated stands (identified as single point locations) were converted to an area assuming the average tree density is 307 trees/hectare and expanded by 3 times to account for the intermittent nature of the treated trees. This is consistent with Ministry of Forests approximate estimates of the affected area (10 MSMA trees = ~0.1 ha affected). We calculated the number of MSMA trees and affected area within 1 km^2 of the nest site and related the percentage of territory affected to the proportion of time spent foraging in MSMA stands.

RESULTS

Woodpecker Debarking

In total, 597 beetle infested trees (reference) and 558 MSMA treated trees were scored for the amount of debarking approximately 10-12 months after infestation. The average debarking score for all 3 years of sampling was lower for MSMA trees than reference trees (Wilcoxon tests, $p < 0.0001$) (Figure 4.1). Debarking of trees by woodpeckers was significantly influenced by MSMA treatment ($p < 0.0001$), tree size (DBH) ($p < 0.0001$), year of infestation

($p < 0.0001$), and decay class ($p = 0.017$) (Table 4.1). Pheromone baiting did not have a significant effect on debarking ($p > 0.05$). The majority (60.4%) of MSMA treated trees were not debarked (index = 0); however, approximately 40% of MSMA trees had some evidence of foraging (index = 1-7) (Figure 4.2). For trees that were marked and followed over a time course, none of the trees were debarked at the time of treatment (first sampling) and woodpecker foraging activity was greatest in the year following infestation between the second and third sampling periods from the fall (November) to the following spring.

Table 4.1: Summary of average debarking scores ± standard deviation for MPB infested Lodgepole pines counted during vegetation surveys at MSMA and reference sites in 2005. Numbers in brackets refer to the sample size i.e. no. of trees counted in that category. GA = green attack (current year infestation), RA = red attack (previous year's infestation), OA = old attack (>2 year old infestation).

Stand type	GA (untreated)	GA (MSMA)	GA (all)	RA	OA
MSMA (n = 8 sites)	2.7 ± 0.8 (196)	1.8 ± 0.5 (109)	2.3 ± 0.5 (305)	3.2 ± 0.7 (25)	4.0 ± 0.5 (19)
Reference (n = 3 sites)	1.6 ± 0.1 (587)	-	1.6 ± 0.1 (587)	2.6 ± 0.2 (59)	3.3 ± 1.5 (16)

Detailed assessments of 3 reference stands and 8 MSMA stands in 2005 revealed that average debarking scores were variable among sites but with no clear explanation as to the cause for such patterns. As part of the vegetation sampling effort which encompassed the core MSMA treatment area, we encountered a large percentage of MPB infested trees that were left untreated (range 12-100% of sample). Within the MSMA sites, the average debarking score was significantly lower for MSMA trees (mean 1.8 ± 0.5) than for trees left untreated (mean 2.7 ± 0.8) (Wilcoxon paired $t_5 = 9.5$, $p = 0.03$). We failed to find any significant correlations between the average debarking score at each MSMA site and variables including the density and number of MSMA trees, density and percentage of GA trees, tree species composition, or abundance of TTWOs, the major species known to debark trees. MPB attacked trees sampled in 2005 revealed a gradual increase in the average debarking score occurred in subsequent years after MPB attack indicating woodpeckers continued to forage on beetle infested trees even after MPB emergence (Table 4.1).

Composition and Abundance of Forest Birds

Point count surveys revealed significant differences in species presence/absence in MSMA stands and reference stands. Woodpecker and other cavity nesting bird densities are shown in Table 4.2. The NOFL ($p = 0.0003$), RNSA ($p = 0.0001$), and DOWO ($p = 0.002$) were all found significantly more frequently (Figure 4.3) and in higher densities (Figure 4.4) in MSMA stands. Only the TTWO was negatively affected by MSMA both in terms of frequency of detection ($\chi^2 = 8.2$, $df = 1$, $p = 0.004$) and relative abundance ($t_8 = -3.1$, $p = 0.02$). However, stand composition did vary among treatment sites (Figure 4.5). There were no differences in the amount of Lodgepole pines among sites; however, the reference sites had a greater percentage of Engelmann spruce and also a greater percentage of MPB infested GA pines than the MSMA sites (Figure 4.6). TTWO abundance was correlated with spruce density ($r = 0.86$, $p = 0.0007$) and with the percentage of GA trees ($r = 0.71$, $p = 0.01$). Therefore, some of the variation in TTWO abundance among MSMA and reference sites was attributed to the availability of spruce and MPB attacked pines. The DOWO ($r = 0.83$, $p = 0.002$) and RNSA ($r = 0.81$, $p = 0.003$) were only significantly correlated with density of Ponderosa pines which was rare in all stands.

Three-toed woodpecker numbers detected during surveys at the sites where insects were collected were strongly and positively related to the abundance of *Dendroctonus* beetles ($r^2 = 0.95$, $p = 0.001$) and to a lesser extent the Scolytidae family ($r^2 = 0.45$, $p = 0.15$) (Figure 4.7). Since *Dendroctonus* collections were comprised largely of MPB and spruce beetle, it is likely that these are the major prey of the TTWO. MSMA sites had lower abundance of Spruce trees and reduced emergence of *Dendroctonus*, particularly MPB, both of which probably influenced the numbers of TTWOs.

Foraging Observations of Woodpeckers

Woodpeckers foraged on a variety of coniferous and deciduous tree species including Lodgepole pine, Douglas fir (*Pseudotsuga menziesii*), Engelmann spruce (*Picea engelmanni*), Ponderosa pine (*Pinus ponderosa*), Trembling aspen (*Populus tremuloides*), and Pacific willow (*Salix lasiandra*) as well as snags, stumps and fallen logs. TTWO were only observed feeding on conifers, primarily Lodgepole pine (66%), while HAWO were recorded foraging on a variety of substrates but mainly Fir (24%), Lodgepole pine (22%) and Aspen (16%) (Table 4.3). Snags were used in 24% and 18% of TTWO and HAWO observations respectively. With respect to Lodgepole pines, both TTWO and HAWO primarily foraged on GA trees or apparently healthy pines (no MPB) with some differences between the species (Table 4.3). Foraging height of infested Lodgepole pines was dependent on the stage of MPB infestation (GA, RA, OA). Woodpeckers were observed foraging lower on the tree bole for GA trees (bottom 1/3 = 59%,

Table 4.2: Average breeding densities (no. birds/ha) of cavity nesting birds surveyed using playbacks at MSMA (n = 7 sites) and reference areas (n = 3 sites) in 2005. Species codes as follows: MOCH=Mountain chickadee, BCCH= Black-capped chickadee, CBCH=Chestnut-backed chickadee, RBNU= Red-breasted nuthatch, DOWO= Downy woodpecker, HAWO=Hairy Woodpecker, TTWO= Three-toed woodpecker, PIWO=Pileated woodpecker, NOFL= Northern Flicker, RNSA = Red-naped sapsucker.

	MOCH	BCCH	CBCH	RBNU	DOWO	HAWO	TTWO	PIWO	NOFL	RNSA
All Sites	0.251	0.009	0.001	0.154	0.012	0.016	0.036	0.018	0.082	0.083
MSMA Sites	0.261	0.012	0.000	0.178	0.018	0.018	0.022	0.018	0.111	0.106
Reference Sites	0.228	0.003	0.002	0.098	0.000	0.011	0.068	0.020	0.016	0.029

Table 4.3: Proportion of first sighting observations (n = 176 records) for foraging substrates that were used by Hairy (HAWO) and Three-toed (TTWO) woodpeckers in 2004 and 2005 at sites throughout the Cascades Forest District. The distribution of HAWO and TTWO foraging observations on Lodgepole pines are further classified by level of Mountain Pine Beetle attack (GA = green attack (current year infestation), RA = red attack (previous year's infestation), OA = old attack [>2 year old infestation]).

Foraging Substrate	% of observations	
	HAWO	TTWO
Live trees		
Lodgepole pine	22.8	65.9
Douglas fir	23.5	4.9
Trembling aspen	15.4	-
Ponderosa pine	4.4	-
Engelmann spruce	3.7	4.9
Pacific willow	0.7	-
Snags		
Fir snag	7.4	7.3
Lodgepole pine snag	5.1	9.8
Spruce snag	-	7.3
Aspen snag	5.9	-
Other		
Stump/Log	10.3	-
Ground	0.7	-
Lodgepole Pines		
GA	31.7	58.1
RA	2.4	3.2
OA	22.0	6.5
healthy	43.9	32.3

middle = 17%, top 1/3 = 24%) and higher on the bole of OA trees (bottom 1/3 = 9%, middle = 27%, top 1/3 = 64%) indicating a difference in foraging strategy and availability of prey species.

The use of MSMA stands by radio-tagged woodpeckers during the breeding season was highly variable among individuals and species. HAWO and TTWO spent on average 13% and 23% (range 0-66%) of their time respectively in MSMA stands (Table 4.4). This is despite the fact that MSMA stands only comprised on average 1-2% of their core home range habitat (1 km2). In contrast to the point count surveys which did not detect any difference between HAWO and TTWO frequency of occurrence in MSMA stands, radio-tagged TTWO occupied MSMA stands more frequently than HAWO ($\chi 2 = 9.7$, df = 1, $p = 0.002$). There was no effect of MSMA stand use by sex ($\chi 2 = 2.3$, df = 1, $p = 0.1$) or by breeding phase ($\chi 2 = 0.07$, df = 1, $p = 0.8$). However, we noticed many birds moved greater distances from the nest site post breeding (see individual bird maps, Appendices 3a-3m). Although not statistically significant, there was a positive trend towards birds spending more time by days ($r = 0.53$, $p = 0.06$) or by minutes ($r = 0.44$, $p = 0.13$) foraging in MSMA stands if a larger percentage of their territory was affected by MSMA.

Table 4.4: MSMA stand use by radio-tagged Hairy (HAWO) (n = 10) and Three-toed (TTWO) woodpeckers (n = 4) followed over the breeding seasons in 2004 and 2005. MSMA stand use is shown by percentage of time (days observed or minutes observed). Table also shows the average number of trees and area affected from MSMA treatments within past 2 years and within 1km^2 of the nesting site.

Species	% of time in MSMA		# MSMA trees	Area affected by MSMA (ha)	% of 1 km^2 territory affected by MSMA
	by days	by minutes			
HAWO	9.0 %	13.3 %	94	0.92	0.9 %
TTWO	22.8 %	23.2 %	201	1.96	2.0 %

DISCUSSION

Woodpecker foraging behaviour and MSMA tree/stand use

We observed woodpeckers foraging on a variety of substrates. TTWO foraged exclusively on conifers, whereas HAWO displayed a more varied foraging strategy. With respect to foraging on Lodgepole pine, both species fed heavily on GA trees presumably taking live MPB larvae and pupae. Our studies of insect emergence (Chapter 3) revealed that many of the stands also had other bark beetles present including spruce, fir and Lodgepole pine beetles. Therefore, woodpeckers likely foraged on non-MPB trees because of the abundance of other beetle species present.

We observed that woodpeckers foraged primarily on Lodgepole pines that had been recently attacked (GA) or on healthy trees, and to a lesser extent on old attack trees (OA). Foraging on OA trees appears to be reduced since the MPB emerges after one year and the current beetle outbreak is a major food source for woodpeckers. However, OA pines including MSMA treated trees that are left standing are typically colonized by secondary woodborers, and could present a continued source of MSMA exposure to forest birds that feed on them. Debarking evidence shows a gradual increase in woodpecker foraging on MPB infested trees over a period of years confirming woodpeckers use these trees even after emergence. We also directly observed woodpecker foraging on OA trees; both treated and untreated, but primarily in the top third of the tree. Therefore, it is likely that woodpeckers are feeding on other secondary insects (i.e. *Ips spp.*) with lower MSMA exposure. Data from the literature shows the translocation ability of MSMA is limited, resulting in reduced arsenic residues in the phloem of the upper bole (Machlauchlan *et al.* 1988a,b). Our data further confirms that *Ips* and other insects have lower arsenic residues than the MPB. Therefore, the current risk for MSMA exposure to woodpeckers feeding on OA trees is present but relatively low. However, future studies should focus on sampling beetle and insect prey from old MSMA trees and monitoring woodpecker use of these trees during the post beetle epidemic, when they are likely to become a more important food source.

We used a combination of methods (debarking scores, radio telemetry, foraging observations, and bird surveys) to evaluate woodpecker use of MSMA stands and the extent of foraging on MSMA versus untreated MPB attacked trees. The use of debarking indices is a simple tool to evaluate woodpecker foraging and to track the timing of MPB predation. Although woodpecker foraging of MSMA trees was significantly lower than non-treated trees, we determined there was still appreciable predation of beetles on treated trees primarily in late

winter and spring. This is likely because MSMA frequently does not kill the entire beetle brood, and larvae were able to survive arsenic concentrations in excess of 300 ppm (Chapter 1). Routine monitoring of MSMA treated trees in the Merritt Forest District in 2004 through random felling and bark removal to assess beetle mortality indicated MSMA killed on average 53% (range 10-100%) of individual beetle broods (MoF unpublished data). Although the pesticide treatment typically reduces the density of the beetle brood (Chapter 3), MSMA trees continue to be an attractive food resource for woodpeckers.

We evaluated forest bird occupancy and abundance in MSMA and reference stands to determine if MSMA treatments altered the use patterns by breeding birds. Densities and frequency of detections of certain species such as the NOFL, RNSA and DOWO were actually higher in MSMA stands, whereas the TTWO was found at lower densities and less frequently in MSMA areas. Radio telemetry data showed that TTWO were foraging regularly in MSMA stands but likely at a lower rate than would be predicted by the survey data at untreated sites. The reduced occurrence and density of TTWO in MSMA stands was attributed in part to the stand composition of the selected survey sites particularly the abundance of spruce and GA trees. However, as seen in previous studies, the TTWO is highly sensitive to changes in bark beetle abundance and its densities directly reflect the availability of this prey source (Fayt *et al.* 2005).

Breeding Hairy and Three-toed woodpeckers are constrained by territorial behaviour (Leonard 2001, Jackson et al. 2002) and most of our radio-tagged birds did not have access to both MSMA and untreated beetle infested stands within their territories. Therefore, comparisons of the occupancy patterns between survey data (at separate MSMA and reference sites) and telemetry data (within a territory) are inappropriate. For radio-tagged HAWO and TTWO breeding in the Merritt Forest District, we determined MSMA stand use was greater than what was proportionally available. Individual radio-tagged HAWO and TTWO also spent more time foraging in MSMA stands when the territory contained a larger fraction of area affected by MSMA. Woodpeckers occupying MSMA stands likely spent some time feeding on many of the GA trees that we detected as untreated. However, breeding territories which are situated within or proximate to high density MSMA treatment areas will predispose the birds to feeding on MSMA trees. Given the two species we studied here, the TTWO appears to be at higher risk than the HAWO for consuming contaminated beetles since its numbers are associated more closely to beetle infestations, it feeds more frequently on MPB attack Lodgepole pines, and it was found more commonly using MSMA stands within its territory.

Effect of MSMA on breeding woodpeckers

MSMA has the potential to affect woodpecker populations either directly through toxic action or indirectly by reducing the food supply. Both mechanisms can potentially influence survival and reproduction. Although we are uncertain as to whether MSMA has an effect in reducing woodpecker numbers through direct toxicity, we have evidence that MSMA may have indirect effects to the TTWO by reducing the food supply and possibly altering its use of treated stands. TTWO abundance was directly related to the abundance of *Dendroctonus* beetles at the stand level. Given the observed reduction in bark beetle abundance at MSMA sites, woodpeckers foraging in MSMA stands would be expected to have a reduction in food supply that would be similar to the effects of harvesting. Goggans *et al.* (1989) found higher nest success in unlogged (58%) verses logged (33%) stands infested with MPB in central Oregon. Furthermore, they found that all the nests that failed during the incubation stage were located in logged stands. All (100%) of our HAWO and TTWO nests that were followed in 2005 (n = 7) and 86% (n = 7) nests in 2004 were successful in fledging at least one young. However, most nests had low reproduction rates with 1 fledging/nest. This is similar to findings of Steeger and Dulisse (1997) in the Deer Creek watershed, B.C. which is also under heavy logging pressure to remove MPB infested trees. Cumulative anthropogenic stressors in our study area from extensive salvage logging to remove beetle infested trees in combination with MSMA treatments could potentially compound the impact of reduced food supply to breeding woodpeckers. Sublethal effects have been shown in laboratory MMAA dosing studies of passerines which included significant mass loss of adult birds and mortality and reduction in growth of nestlings (Albert 2006). A reduction in food availability could potentially be occurring in these study areas thereby amplifying the toxic effects of MSMA to adult and nestling woodpeckers.

Bark beetle management and woodpecker conservation

The ecological role of woodpeckers, particularly those of the genus *Picoides*, in regulating beetle populations has been well documented in the literature (Baldwin 1960, Goggans *et al.* 1989, Steeger and Dulisse 1997, Steeger *et al.* 1998, Fayt *et al.* 2005). The woodpecker guild is considered one of the most important predators of insect pests throughout North America (Buckner 1966). Woodpeckers will frequently aggregate in beetle infested areas showing a numerical response to bark beetle outbreaks (Rust 1929, Otvos 1965, Koplin and Baldwin 1970, Koplin 1972, Kroll and Fleet 1979, Villard and Beninger 1993). They have also been observed to increase the amount of bark beetles in their diet as a functional response to the beetle epidemics (Koplin and Baldwin 1970, Koplin 1972, Crocket and Hansley 1978). Kroll *et al.* (1980) provided recommendations for managing beetle infested stands and cautioned

against the use of large scale salvage logging to remove all damaged trees because of the potential for reducing woodpecker populations and ultimately causing increases in beetle populations. Current forest practices which include large scale harvesting in combination with MSMA applications will reduce the available habitat in many of the MPB infested areas within the study area and place insectivorous predators at increased risk of exposure. Therefore, current beetle management practices that use MSMA and large scale salvage logging have the potential to limit woodpecker populations through a combination of effects including a reduction in food supply, an increased susceptibility to exposure and potential direct toxicity.

Figure 4.1: Average debarking score (± SD) for MSMA treated and untreated green attack trees randomly sampled in 2002, 2003 and 2005. Numbers in bars represent sample sizes (number of trees).

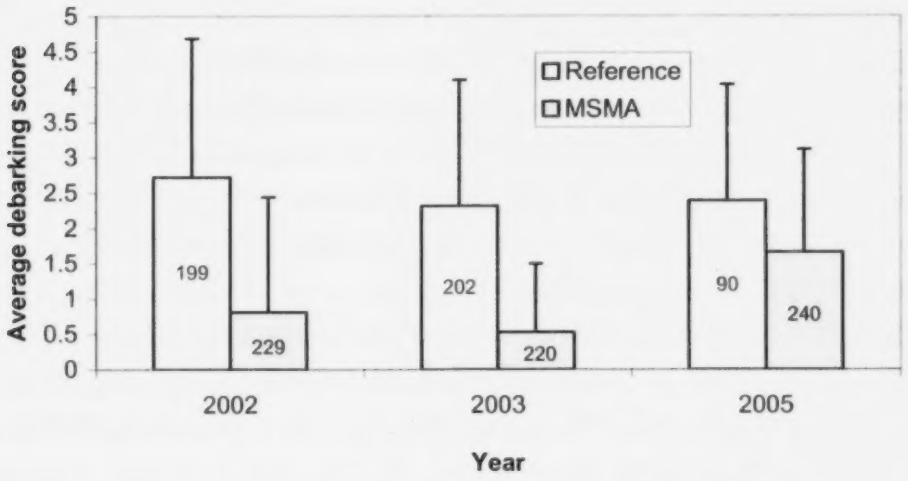


Figure 4.2: Percent of trees identified (reference and MSMA) in each debarking category indicating level of foraging activity by woodpeckers 10-12 months after infestation and/or treatment. Data collected over 3 years (2002, 2003, 2005) using 597 reference trees and 558 MSMA trees. See methods for explanation of debarking scale.

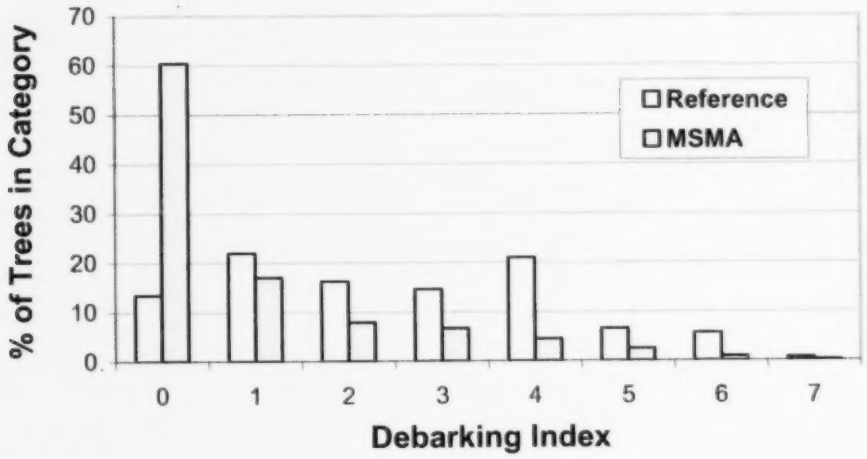


Figure 4.3: Frequency of species detections (proportion of surveys detected) from playback surveys at MSMA and reference sites in Cascades Forest district during the breeding season in 2005. Asterisks above bars indicate significant differences existed among MSMA and reference sites.

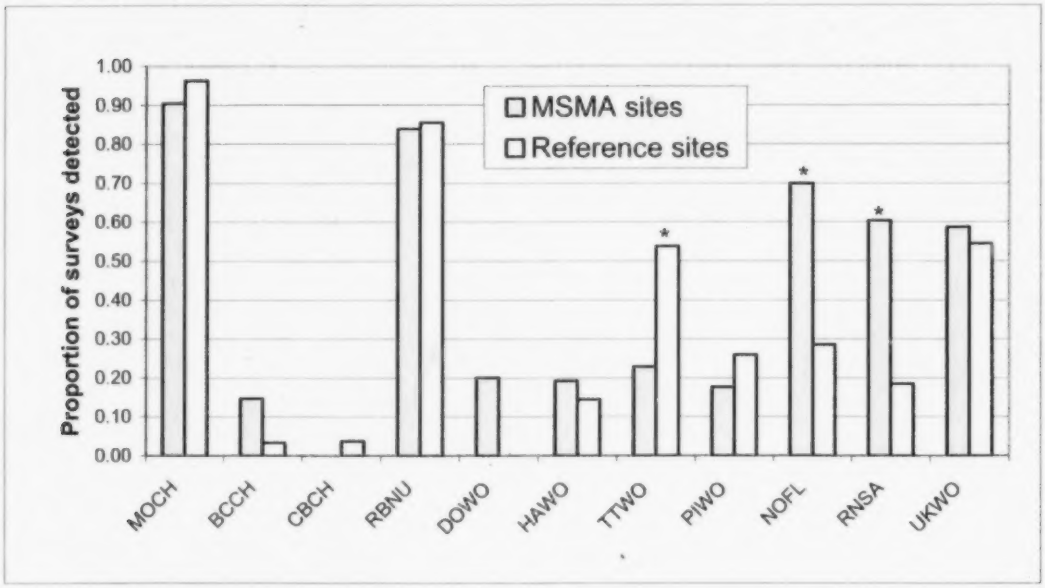


Figure 4.4: Mean density (no. birds/ha ± SD) of woodpeckers counted during the breeding season in 2005 during playback surveys at MSMA (n = 7 sites) and reference (n = 3 sites) areas in Cascades Forest district. Asterisks above bars indicate significant differences existed among MSMA and reference sites.

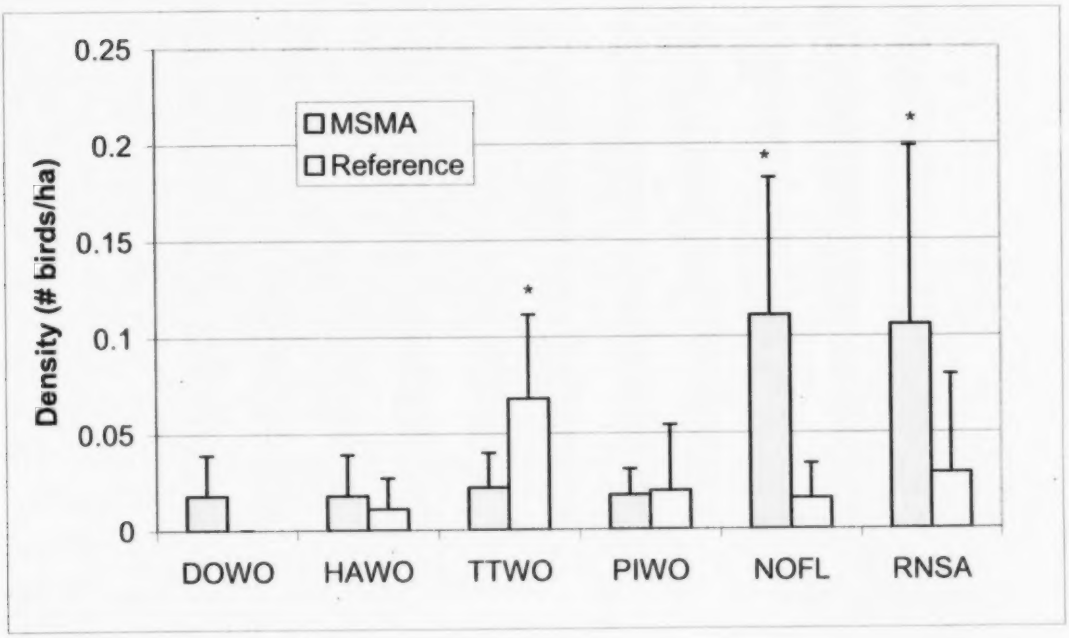


Figure 4.5: Tree species composition in selected MSMA and reference sites in the Cascades Forest District that were sampled during vegetation surveys in 2005.

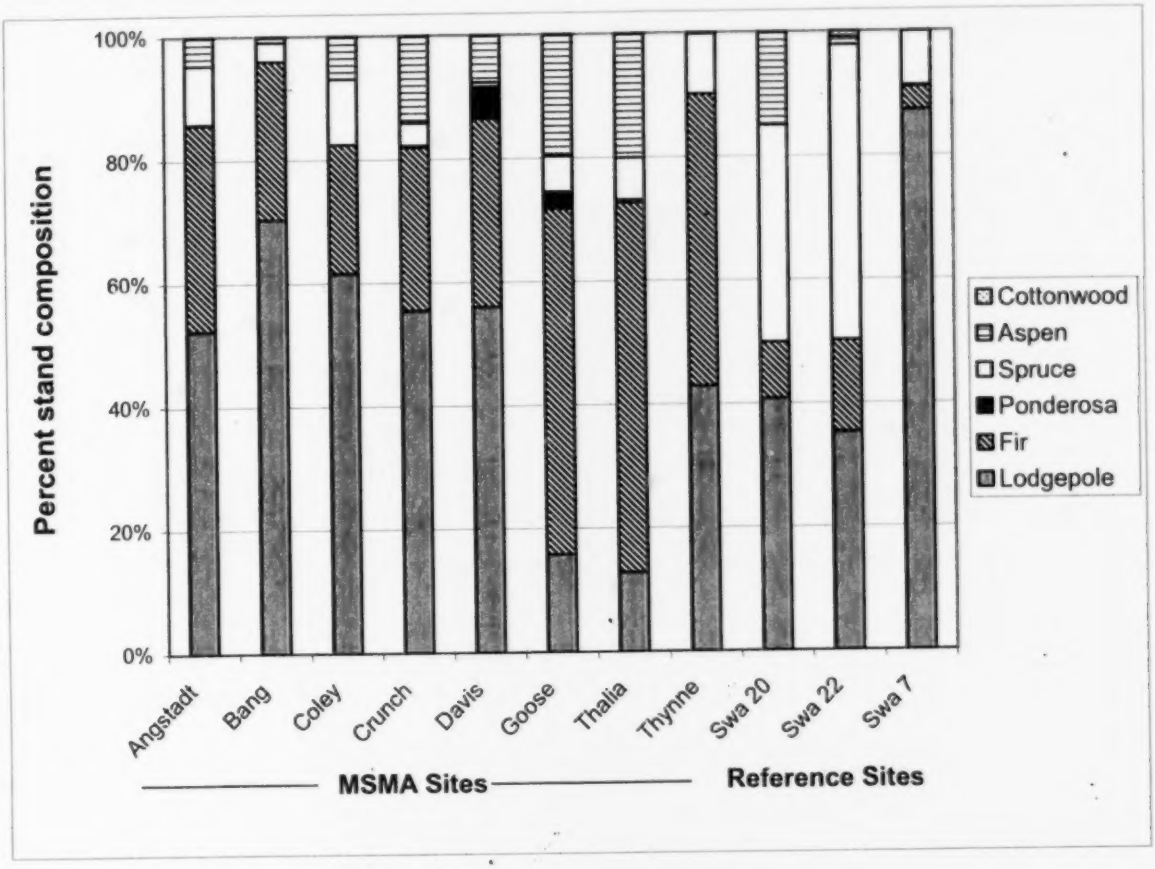


Figure 4.6: Composition of selected MSMA stands and reference stands in the Cascades Forest District that were sampled during vegetation surveys in 2005 shown as percentage of green attack (GA), red attack (RA), and old attack (OA) Lodgepole pines.

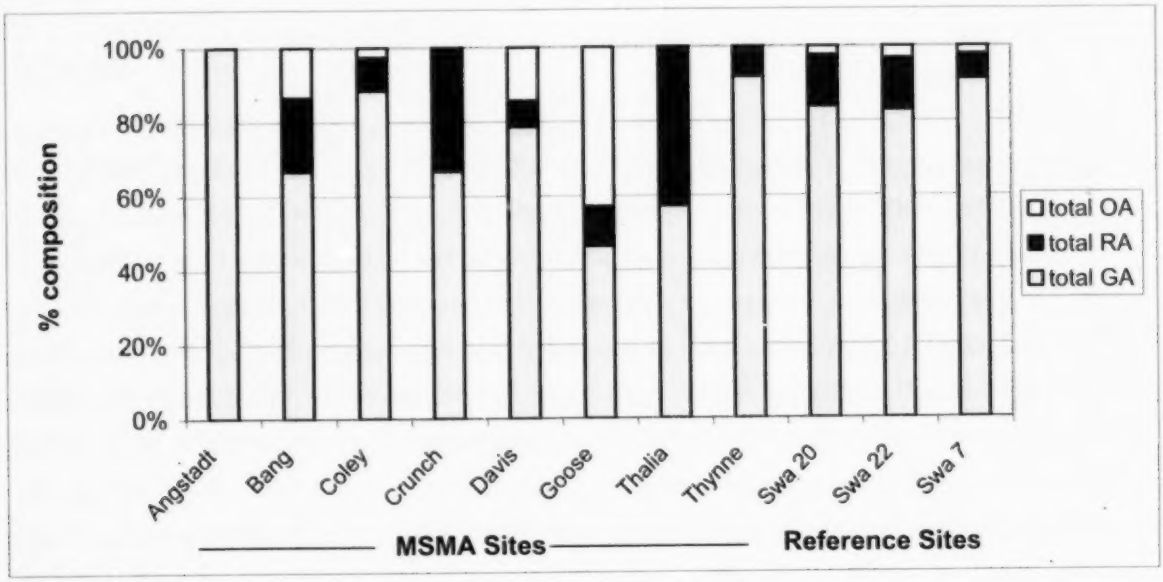
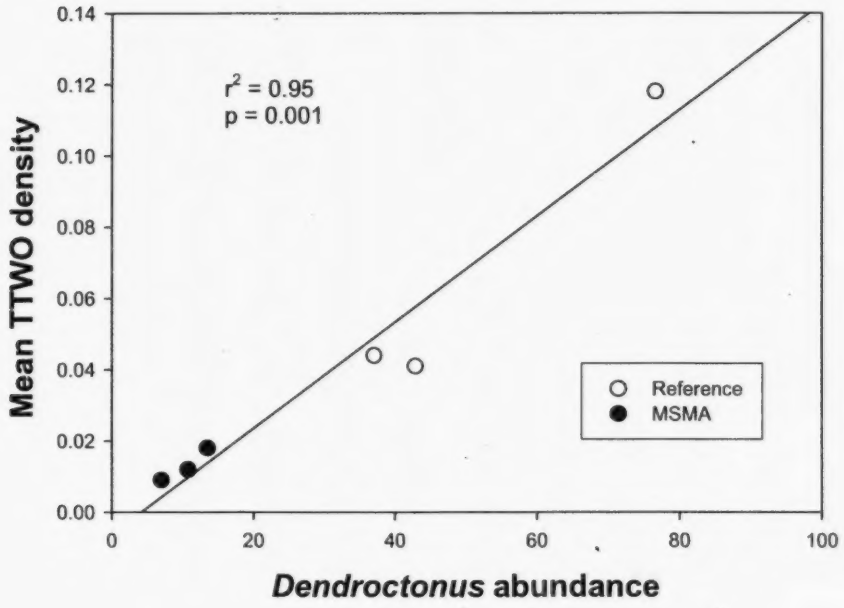


Figure 4.7: Relationship between the total insect trap abundance of *Dendroctonus* beetles and the mean density of Three-toed woodpeckers occupying MSMA (n = 3) and reference (n = 3) stands in the Cascades Forest District, 2005.



CHAPTER 5:

SPATIAL ANALYSIS AND MSMA USE PATTERNS IN THE MERRITT FOREST DISTRICT, 2000-2004

METHODS

Geographic Information Systems (GIS) Analysis

MSMA use in the Cascades (Merritt) Forest District was mapped using ArcMap® version 9 (ESRI, Redlands, California) for the most recent treatment years in 2002, 2003 and 2004 using unpublished data provided by the Ministry of Forests (Merritt office). No further MSMA treatments were applied in 2005 and are not anticipated in the near future. The Ministry of Forests data contained information on forest health surveys conducted in 2002, 2003, and 2004 for sites throughout the Merritt Forest District, which included a single Global Positioning System (GPS) coordinate for each treated site and the number of MSMA trees treated within each site. We converted the GPS point locations for individual MSMA treated stands containing variable numbers of MSMA trees to a buffered affected area assuming the average tree density is 307 trees/hectare. This assumption is based on our vegetation data collected for 10 sites in the Merritt Forest district in 2005 and represents a minimum area affected if all the treated trees are clustered. In reality, the affected areas are likely 2-5 times larger than this estimate because MSMA trees are more spread out. Thus, MSMA treatment areas were expanded by 3 times to account for this. This is consistent with B.C. Ministry of Forests estimates of the affected area (10 MSMA trees = ~0.1 ha affected).

Estimates of Total MSMA Use in Cascades Forest District

We used data from the B.C. Ministry of Forests on actual numbers of treated trees for 2002-2004 and from the B.C. Ministry of Water, Land and Air Protection on the amount of MSMA active ingredient (a.i.) used and/or number of treated trees for 2000 and 2001 in the Cascades Forest District. Given that the prescribed MSMA application rate is 1 mL of Glowon® (0.32 kg MSMA/L water and additives) per 2.5 cm tree circumference (United Agri Products Canada, Glowon product label), we calculated application rates to be on average 10.88 g MSMA (a.i) per tree. This calculation assumes average tree diameter of MSMA trees = 27 cm, circumference = 85 cm which is based on our measurements of >500 MSMA trees treated in recent years.

RESULTS AND DISCUSSION

While the mapping information presented here is limited to our study area and study years 2002-2004, the intention is that this would be a model for future mapping of MSMA treatment sites in all years and forest districts within the province. MSMA was used over a large area within this forest district with some localized areas of intensive multi-year applications (Figure 5.1).

Our estimate of how much MSMA is applied to each tree (10.88 g MSMA/tree) was within 5% of the actual use estimates (11.43 g MSMA/tree) using data available from the BC MWLAP which provided both the number of treated trees and the amount of pesticide used in 2001. In the Merritt Forest district alone, a substantial proportion of the province's total MSMA use occurs in this region (Figure 5.1). From 2000-2004, over 66 000 cumulative trees were treated amounting to 737 kg of MSMA (Table 5.1). However, note the year's 2003 and 2004 had a substantial decrease in MSMA use compared to the previous years 2000-2002 (MWLAP/MoF unpublished data).

Table 5.1: Summary of MSMA applications in the Cascades (Merritt) Forest District from 2000-2004.

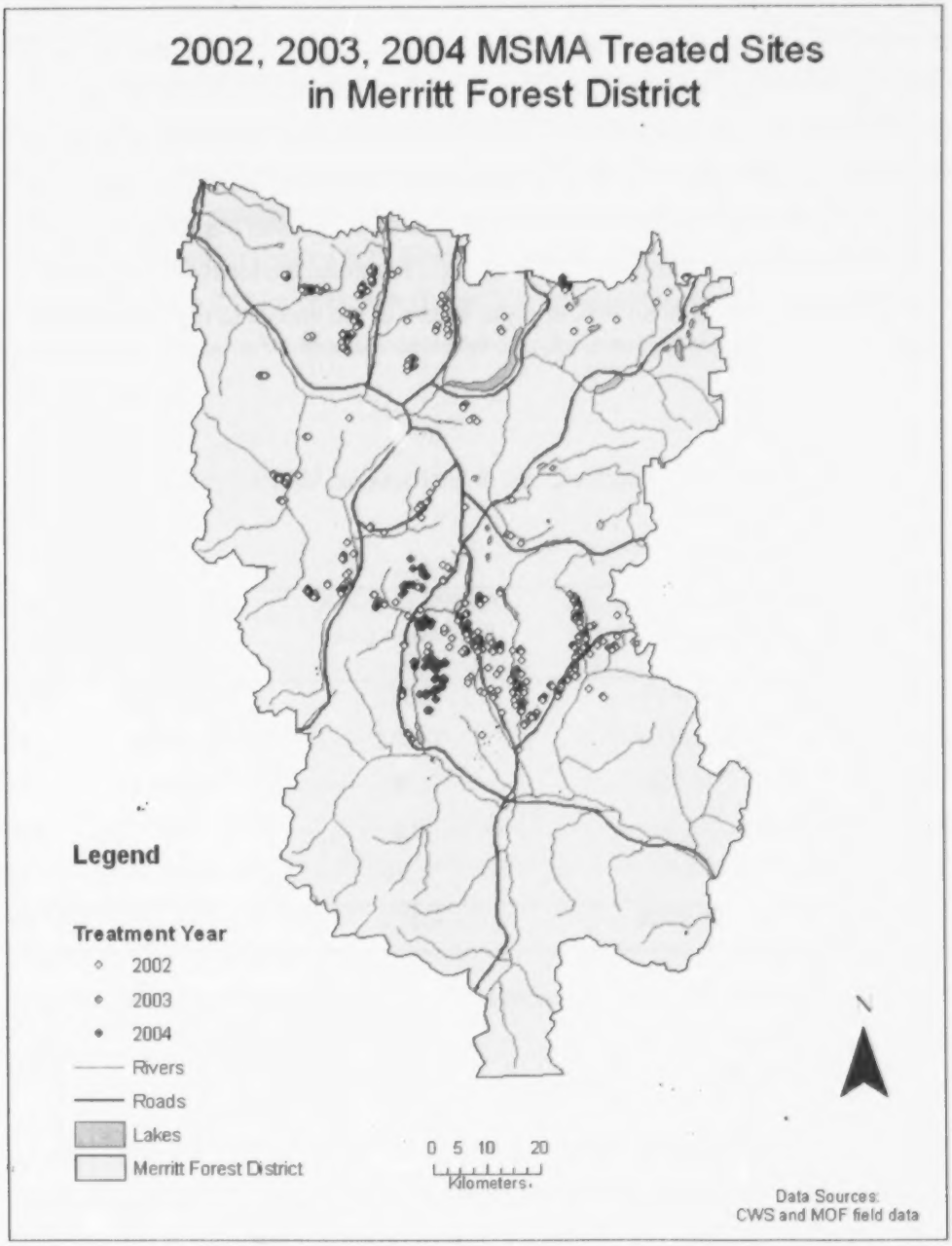
Year	No. MSMA trees	Amount MSMA (a.i.) used (kg)	Amount Glowon® product used (L)	Effective area treated (ha)
2000	21 020[c]	228.7[a]	714.7	228.23
2001	30 447[a]	348.0[a]	1035.2	297.53
2002	12 406[b]	135.0[c]	421.8	121.23
2003	362[b]	3.9[c]	12.3	3.54
2004	1 954[b]	21.3[c]	66.4	19.09
Total	**66 189**	**736.9**	**2302.7**	**669.62**

[a]source: BC Ministry of Water, Land, Air Protection
[b]source: BC Ministry of Forests
[c]calculated (see methods)

Although the number of treated trees in any given year or forest district appears small, the trees are not typically harvested and therefore the total amount of MSMA accumulates in the environment each year. Information on provincial MSMA use reported to the B.C. Ministry of Water, Land and Air Protection (MWLAP) from Pesticide Use Permits and Pest Management

Plans indicated that in the past 10 years (1994-2003), approximately 5,080 kg of MSMA were cumulatively used in British Columbia (D. Cronin, MWLAP, *pers. comm.)* amounting to some 467,000 trees. This figure is likely underestimated since there were several regions with no data available for one or more years. MSMA applications in British Columbia have been estimated to be on average 750 kg annually (Dost 1995). If this estimate is correct, we would expect that approximately 69 000 trees were treated annually in British Columbia and that the 10 year cumulative estimate would be closer to 700,000 trees. Since MSMA has been used for approximately 20 years and the trees are not typically harvested, the cumulative numbers of MSMA trees throughout the region has led to a substantial area that remains contaminated. While we were able to estimate the number of MSMA treated trees, with the exception of the most recent years, most of those trees are not mapped. Therefore, the location and fate of previously treated MSMA trees is unknown, and the extent of the impact to the environment remains unclear.

Figure 5.1: Map of the Cascades (Merritt) Forest District showing location of all MSMA sites for the last 3 years (2002-2004) of use.



CHAPTER 6:

PRELIMINARY RISK ASSESSMENT OF WOODPECKERS BREEDING IN PROXIMITY TO MSMA STANDS

METHODS

We conducted a preliminary ecological risk assessment designed to assess if arsenic from MSMA applications (form of MMAA) could adversely affect non target wildlife species, specifically, woodpeckers and other cavity nesting insectivorous birds breeding in close proximity to recent MSMA treatment areas in the Merritt Forest District. The information provided to model the exposure assessment is based on data available in this report and from data in the literature. The assessment of effects is based on studies conducted by Albert (2006). Data for the assessment will be separated by species (Hairy and Three-toed woodpeckers), ages (nestling/adult) and sexes.

Contaminant and Receptors of Concern

For this assessment, we considered only MSMA as a contaminant of concern in the Merritt Forest district. Measurements are provided as total arsenic. Bark beetles from MSMA trees were typically 90-97% monomethylarsonic acid (MMAA) which is the predominant form of MSMA at gastrointestinal pH (Chapter 1). Although a variety of cavity nesting bird species are present in our study area, we selected the Three-toed and Hairy woodpecker as the most important and highest risk target species/populations for toxic exposure to MSMA. Other primary and secondary cavity nesting birds are also at risk for consuming contaminated prey, but likely at lower relative concentrations. Table 6.1 shows the list of avian wildlife assessment endpoints that are relevant to the area and contaminant of concern. MSMA is hypothesized to potentially impact the foraging habitat of cavity nesting forest bird populations. The species listed are possibly at risk because they are relatively common in the area, their diet is made up primarily of animal origin, and/or they are known to show a functional and numerical response to bark beetle outbreaks.

Exposure assessment

The exposure assessment quantitatively estimates potential exposure of the selected receptors to MSMA. An exposure (dose) model incorporating woodpecker natural history information and characteristics (including diet composition, ingestion rates, body weights, and foraging ranges) was developed to evaluate exposure. The identified media of concern are the

Table 6.1: Cavity nesting forest bird species encountered during our study and proportion of diet that is animal origin (Steeger *et al.* 1998).

Species	Diet (% animal origin)
Primary cavity nesters (Woodpeckers)	
Three toed woodpecker	89-96
Black backed woodpecker	>90
Hairy woodpecker	77-93
Downy woodpecker	76-97
Northern Flicker	61-95
Red naped sapsucker	50
Pileated woodpecker	73-75
Secondary cavity nesters (weak excavators)	
Mountain chickadee	75-98
Chestnut backed chickadee	65
Black-capped chickadee	70
Red-breasted nuthatch	70-88
Brown creeper	>85

whole body prey items (bark beetles) contained within MSMA treated trees. Ecological receptors may be exposed to arsenic through three major pathways: ingestion, dermal contact, and inhalation. In the case of woodpeckers occupying MSMA stands, we assumed the only pathway with appreciable risk would be through ingestion of bark beetle prey items and possibly ingestion of contaminated sap and phloem. We have limited our assessment to consumption of bark beetles because there is neither data available on arsenic levels in sap or phloem nor estimates of ingestion rates for these items. Birds are assumed to be at no risk for exposure from water or soil as MSMA does not accumulate appreciably in the surrounding environment (Norris *et al.* 1983) and woodpeckers are unlikely to be ingesting any water or soil that would be contaminated with MSMA. We have not included dermal contact or inhalation in our assessment.

To assess exposure to woodpeckers, a dose assessment model was developed to estimate the daily dose of arsenic to woodpeckers from the ingestion of contaminated MPB. The following dose model was used to assess daily exposures of contaminants to woodpeckers and to characterize exposure:

$$\text{Daily Dose} = \frac{(C_{food} * IR_{food}) * AUF}{BW} \qquad \text{(Equation 1)}$$

Where:

Dose = daily dose resulting from ingestion (mg/kg/day)

C_{food} = concentration of arsenic in prey (mg/kg dry weight)

IR_{food} = estimate of daily ingestion rate of food tissue (kg/day dry weight)

AUF = area use factor (between 0 and 1)

BW = body weight (kg)

Data used for the exposure assessment are summarized in Table 6.2. We evaluated dose at 2 levels: moderate exposure and high exposure. Concentrations of total arsenic detected in woodpecker prey represented levels detected in MPB adults and larvae from MSMA trees at 50 mg/kg (moderate exposure) and 350 mg/kg (high exposure). Area use factors (average and highest use) were derived from our radio telemetry observation data (Chapter 4). We assumed the amount of MPB in the diet was in direct proportion to the amount of time spent feeding in MSMA stands. Body weights for Hairy and Three-toed woodpeckers collected in British Columbia were taken from the literature (Leonard 2001, Jackson *et al.* 2002). Allometric food ingestion rates of insectivorous birds were calculated from equations provided by Nagy (2001) and entered into the model for males, females and nestlings separately. Given that nestlings are developing over the course of ~30 days in the nest, their body mass and food intake rates change dramatically over time. However, the assessment requires selection of a time period to evaluate risk. Fledgling woodpecker body mass is reported as equivalent to adults at the time of fledging and shows a sigmoidal pattern of development from hatching to fledging (Hadow 1976, Weathers *et al.* 1990). Weathers *et al.* (1990) also determined daily metabolic rates of Acorn woodpecker (*Melanerpes formicivorus*) nestlings stabilized at 82% of the adult level by 3 weeks of age (1 week prior to fledging). Therefore, we estimated body mass of Hairy and Three-toed woodpecker nestlings at approximately 3 weeks of age to be equal to the lower weight females (smallest) and food intake rates to be 20% lower than adults.

Risk characterization

Potential risk was characterized by comparing the modeled dose estimate to specific toxicity reference values (TRV) taken from Albert (2006) for adult and nestling Zebra finches orally dosed with MMAA. We did not apply any interspecies extrapolation factors to account for possible differences in sensitivity between the Zebra finch and woodpeckers. That would

Table 6.2: Summary of data used for exposure assessment model for Hairy and Three-toed woodpecker adult and nestlings exposed at moderate and high rates. Parameters used for the model included body weights (BW), total arsenic residues in food (C_{food}), food ingestion rates (IR), and area use factors (AUF).

Species Age/Sex	BW(kg)	C_{food} (mod) (mg/kg/d)	C_{food} (high) (mg/kg/d)	IR(food) (kg/d)	AUF (mod)	AUF (high)
HAWO adult male	0.0850	50	350	0.0124	0.13	0.60
HAWO adult female	0.0757	50	350	0.0114	0.13	0.60
TTWO adult male	0.0583	50	350	0.0095	0.23	0.66
TTWO adult female	0.0520	50	350	0.0088	0.23	0.66
HAWO nestling	0.0757	50	350	0.0091	0.23	0.66
TTWO nestling	0.0520	50	350	0.0070	0.23	0.66

have provided a more conservative assessment; however, we have no estimate of the degree of uncertainty between these species. The risk characterization should be evaluated with caution, therefore, and a more conservative approach may be necessary. Reference values represent the lowest observable adverse effect level (LOAEL) for birds dosed at relevant concentrations. Albert (2006) found significant mass loss in adult Zebra finches dosed at 24 and 72 mg/kg/day and a reduction in growth (tarsus measurement) for nestling Zebra finches dosed at 8 and 12 mg/kg/day. No observable adverse effect (NOAEL) was recorded in the adults at 8 mg/kg/day or the nestlings at 4 mg/kg/day. Although, the nestling study detected complete mortality at 12, 24, 36 and 72 mg/kg/day in two pilot studies, a repeated study with larger group sample sizes and reduced dosage levels found low mortality rates (3/23 birds) at 12 mg/kg/day. Given the uncertainty around the dosage level which caused mortality, we did not use that measure as an endpoint for our TRV. Since most of the arsenic present in bark beetle samples consisted of 90-97% MMAA, we assumed that a TRV from songbirds dosed with MMAA would be directly comparable to woodpeckers consuming contaminated beetles (measured as total arsenic).

The estimated daily doses for male and female Hairy and Three-toed woodpeckers were calculated (Equation 1) for MMAA, which were then compared to the TRVs to establish a series of hazard quotients using the following equation:

$$HQ = dose/TRV \qquad \text{(Equation 2)}$$

The dose estimates were used to derive a series of hazard quotients (HQs), using the average exposure dose estimate and high exposure dose estimate. These were compared to the NOAEL (HQ_{NOAEL}) and the LOAEL (HQ_{LOAEL}). Assuming that woodpecker sensitivity is equal to

or less than the Zebra finch which was used to calculate the TRV, if the dose is lower than the low TRV (*i.e.*, HQ_{NOAEL} <1), it is likely that little or no risk is present from the contaminant. When the dose exceeds the low TRV (*i.e.*, HQ_{LOAEL} >1), it indicates that further evaluation may be warranted. Following EPA's 8-step Ecological Risk Assessment process, there are three possible outcomes: (1) it is adequate to conclude that the site poses no unacceptable risks to ecological receptors; (2) the conclusions are inconclusive and the ecological risk evaluation should continue; and (3) a potential for adverse ecological effects is indicated and a more thorough analysis is warranted (EPA 1998).

RESULTS AND CONCLUSIONS

Exposure assessment

Woodpeckers breeding in MSMA stands were exposed to MSMA at moderate concentrations under average conditions (consuming bark beetles with ~50 ppm total arsenic and moderate use of MSMA stands). The Three-toed woodpecker generally had higher daily doses than Hairy woodpeckers given their more specific bark beetle diet and smaller average body weights. The effect of sex and age had only a minor effect on overall exposure. Woodpeckers occupying MSMA stands under high exposure conditions when taking into account the upper range of arsenic contaminated beetles and area use factors would be expected to have dramatically higher daily doses (~30x higher). In reality, the daily dose to woodpeckers is likely to be highly variable both spatially and temporally with some birds receiving large doses infrequently and low to moderate doses more commonly. Sites with a greater proportion of MSMA trees in the bird's territory were found to have higher occupancy/use (Chapter 6) which would predispose them to increased risk of exposure.

Risk characterization and assessment

The risk characterization combines the exposure and effects assessments to provide an estimate of the potential risks to receptors. The preliminary risk assessment evaluated exposures to adult and nestling Hairy and Three-toed woodpeckers from MSMA. The results are summarized in Table 6.3. Woodpecker exposures (adults and nestlings) at the moderate doses were all below the TRV_{NOAEL} indicating little or no risk for toxicity. However, the HQ_{NOAEL} and HQ_{LOAEL} for all groups using the higher dose exposure estimates exceeded 1.0, indicating high risk for toxic effects to occur. In general, the Three-toed woodpecker had higher daily exposure and therefore, had higher HQ values. Exceedance estimates were also notably higher for nestlings compared to adult birds which are consistent with reported higher sensitivities of young developing animals.

Table 6.3: Summary of modeled daily dosage and ecological risk (toxicity reference values (TRV) and hazard quotients (HQ)) for adult and nestling Hairy and Three-toed woodpeckers occupying MSMA stands. Highlighted values are the hazard quotients found to exceed 1, which identifies potential risk for toxic effects.

Estimated daily dose (mg/kg/d)		**TRV (mg/kg/d)**		**HQ Moderate exposure**		**HQ High exposure**	
Moderate	High	NOAEL	LOAEL	HQ_{NOAEL}	HQ_{LOAEL}	HQ_{NOAEL}	HQ_{LOAEL}
0.95	30.58	8	24	0.12	0.04	3.82	1.27
0.98	31.64	8	24	0.12	0.04	3.96	1.32
1.87	37.60	8	24	0.23	0.08	4.70	1.57
1.94	38.89	8	24	0.24	0.08	4.86	1.62
1.39	27.85	4	8	0.35	0.17	6.96	3.48
1.55	31.11	4	8	0.39	0.19	7.78	3.89

The risk assessment results are consistent with blood arsenic data we collected from 3 species of woodpeckers and 2 species of passerines (Chapter 2). Albert (2006) found a dose response relationship with blood arsenic concentrations in Zebra finches orally dosed with MMAA. Our data on blood arsenic values were in the range of values for the 8 and 24 mg/kg/d dose groups where significant mass loss is expected to occur at 24 mg/kg/d. Based on the same study, nestling growth is also expected to be affected by MSMA at doses relevant to what developing woodpeckers in our study area are predicted to be receiving. Therefore, we have sufficient data to show that exposure to MSMA is occurring and that there is potential for sublethal effects including mass loss and reduced growth under current environmental conditions. It can be concluded that based on exposure and effects data available for MSMA, significant risk to woodpeckers occupying MSMA treatment stands is present and further analysis is warranted.

CHAPTER 7:

SUMMARY AND RECOMMENDATIONS

The British Columbia Ministry of Forests has been attempting to contain and prevent large scale infestations of the current MPB epidemic for several years through a variety of methods. The use of pesticides (MSMA) has recently generated concern because of uncertainty about the potential hazards to humans, wildlife, and the environment. This study attempts to answer a number of questions about the exposure of woodpeckers and other forest birds which are the species guild most likely affected by MSMA.

Bark beetles exposed to MSMA were found to significantly accumulate arsenic in the form of monomethyl arsonic acid (MMAA). Many of the MPB larvae can survive MSMA treatment and accumulated up to 327 ppm total arsenic and one sample containing dead larvae contained 700 ppm total arsenic. Several woodpecker species including Hairy, Three-toed and Black-backed woodpeckers are known to prey upon larval, pupal and adult stages of bark and wood-boring beetles with diets often consisting from 60% to 99% beetle larvae by volume (Steeger *et al.* 1998). We were able to confirm that MSMA does effectively reduce the emergence of bark beetles at the stand level; however, many beetle infested trees are missed during the treatment period and developing larvae in treated trees are able to survive high concentrations of arsenic. Therefore, in contrast to previous understanding, MSMA stands do remain attractive to woodpeckers for foraging.

Although predation (debarking) on treated trees was lower than that of reference trees, blood arsenic concentrations in most woodpeckers and other forest passerines sampled were significantly elevated above reported arsenic reference concentrations at an uncontaminated site (0.005-0.02 ppm) (Burger and Gochfeld 1997) and within the range of those levels found in the low and medium dose groups (consuming 8 and 24 mg MMAA/kg bw/day) of adult Zebra finches that were orally dosed (Albert 2006). Blood sampling may not be an ideal medium to monitor exposure to MSMA given the large variation among individuals that was not well correlated to various predictors. Knowing the rapid accumulation and loss of this compound and the uncertainty of the timing of birds feeding on contaminated trees, we suggest the blood arsenic data indicates recent exposure but should not be used for inferring quantitatively the degree of exposure.

By collecting data on the amount of debarking on treated and reference trees throughout the study area and by observing radio-tagged woodpeckers, we were able to confirm that woodpeckers are foraging in beetle infested areas including MSMA stands and specifically MSMA trees. Several radio-tagged woodpeckers, particularly the TTWO in this study, spent a significant proportion of their time foraging in MSMA stands potentially ingesting numerous contaminated beetle larvae. Woodpeckers have been reported to aggregate in beetle-infested areas in unusually high densities as a functional response to increasing prey densities (Koplin 1972). We also confirmed Three-toed woodpeckers respond in this fashion and their densities were directly related to the abundance of *Dendroctonus* beetles (mainly Mountain Pine and Spruce beetle). Since woodpeckers are known to be effective at controlling incipient populations of beetles, and the use of MSMA is typically recommended under these same conditions, MSMA is likely to alter the natural process of insect pest regulation by avian predators. This is potentially exacerbated when harvesting pressure in neighbouring stands further reduces available nesting and foraging habitat. Forest practices that use MSMA and harvesting to reduce beetle densities are subsequently impacting woodpeckers through a combination of effects including a reduction in food supply, an increased susceptibility to exposure and potential direct toxicity. The risk assessment showed that exposure to MSMA is likely occurring and that there is potential for sublethal effects including mass loss and reduced growth under current environmental conditions.

Given the levels of arsenic detected in bark beetles and in avian predators occupying MSMA stands, the intensity of woodpecker foraging activity on MSMA trees, and the toxicity concerns for avian wildlife consuming contaminated beetles, there is concern about potential future use of MSMA and the legacy of MSMA trees being left standing in B.C. forests as existing wildlife habitat. The extent of foraging that occurs on old MSMA trees, although present, appears to be reduced since the MPB emerges after one year and the current beetle outbreak is a major food source for woodpeckers. However, we recommend future studies evaluate arsenic residues in insect samples collected from old MSMA trees that are likely to be colonized by secondary woodborers to identify potential risks. Future monitoring of woodpecker foraging on old MSMA trees would be useful to improve our understanding of how to reduce exposure to MSMA given the current harvesting pressures of untreated trees and the expansive use of MSMA in many areas. Research should focus on the exposure of birds to MSMA during the post beetle epidemic when changes in food supply brought about by a reduction in the MPB may make old MSMA trees a more attractive food source.

Past forestry practices frequently omitted marking and recording MSMA trees and therefore, with the exception of recent treatments, most trees are not mapped and the extent of impact to the environment remains unknown. Our attempt to map the most recent MSMA treatments for the Merritt Forest District may be useful as a model for expanding to the entire province for all treatment years. MSMA treated trees left standing appear to pose the greatest risk to forest birds particularly in areas where extensive harvesting has removed alternative healthy habitat. Felled trees that are left to decay are also fed upon by some species of woodpeckers and used by other wildlife while also contributing to the movement of arsenic into the surrounding environment. Management actions designed to mitigate the potential effects to forest birds from foraging on old MSMA trees should consider the potential negative impacts that cumulative applications of MSMA can have on the forest ecosystem. Given the evidence of exposure of insectivorous birds to MSMA and the demonstrated risk of toxicity, we recommend that no future use of the pesticide should be permitted, and that alternative means of MPB suppression, chemical or otherwise, be implemented. We also recommend that forest management plans should determine on a site by site basis whether old MSMA trees be felled and/or removed as appropriate, and that equivalent wildlife habitat areas be created in compensation.

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APPENDICES

Appendix 1: Details of bark beetle samples collected during 2002-2005 in the Cascades Forest District for total arsenic analysis and arsenic speciation (As(III), As(V), MMAA, DMAA).

Trt Year	Sample ID	Collection Date	Group	Life Stage	Morbitity	Treatment	Collection Height (m)	Debark Index	As(III)	DMAA	MMAA	As(V)	Total Extract (µg/g)	Total As (µg/g)	Extraction Efficiency (%)
2001	A1	2002	Cleridae	larvae	live	Red control	0-2		0.06	0.01	0.0	0.04	0.10	1.62	6.0
2001	A2	2002	Cleridae	larvae	live	Red control	0-2		0.0	0.0	0.0	0.0	0.0	0.08	0.0
2001	B1	2002	MPB	larvae+pupae	live	Red control	0-2		0.0	0.0	0.0	0.0	0.0	0.11	0.0
2001	C1	2002	MPB	adult	dead	Red control	0-2		0.0	0.0	0.01	0.0	0.0	0.09	0.0
2001	C2	2002	MPB	adult	dead	Red control	0-2		0.0	0.0	0.0	0.01	0.0	0.02	0.0
2001	D1	2002	MPB	adult	dead	Red control	0-2		0.0	0.0	0.0	0.0	0.0	0.00	0.0
2001	E1	2002	Wood-boring beetle	larvae	live	Red MSMA	0-2	3+	0.0	0.01	0.17	0.0	0.17	0.22	76.9
2001	F1	2002	MPB	larvae+pupae	live	Red MSMA	0-2	3+	0.0	0.08	2.64	0.0	2.7	11.3	24.1
2001	G1	2002	Other insects	adult	live	Red MSMA	0-2	3+	0.0	0.0	11.8	0.0	11.8	37.0	31.9
2001	H1	2002	MPB	adult	dead	Red MSMA	0-2	3+	1.3	0.6	91.6	1.6	95.0	68.2	139.3
2001	H2	2002	MPB	adult	dead	Red MSMA	0-2	3+	1.1	0.0	25.4	0.9	27.5	57.3	47.9
2002	J1	2002	MPB	adult	live	Green MSMA	0-2		2.3	0.2	74.7	4.8	81.9	82.5	99.3
2002	J2	2002	MPB	adult	live	Green MSMA	0-2		1.8	0.2	24.3	2.8	29.1	55.1	52.9
2002	J3	2002	MPB	adult	live	Green MSMA	0-2		0.3	0.0	20.1	1.3	21.7	40.7	53.4
2002	K1	2002	MPB	adult	live	Green control	0-2		0.0	0.0	0.2	0.0	0.25	0.32	79.8
2002	K2	2002	MPB	adult	live	Green control	0-2		0.0	0.0	0.2	0.0	0.21	0.70	30.0
2001	L1	2002	Ips	adult	live	Red MSMA	0-2	3+	0.1	0.00	0.2	0.6	0.90	0.63	143.5
2002	A1	7/22/2003	MPB	larvae+pupae	live	Red MSMA	0-2	5	0.1	0.2	3.3	0.1	3.7	6.19	59
2002	A2	7/22/2003	MPB	larvae+pupae	live	Red MSMA	0-2	3	0.3	0.4	29.9	0.5	31.6	43.52	73
2002	A3	7/24/2003	MPB	larvae+pupae	live	Red MSMA	0-2	3	0.1	0.02	3.7	0.1	3.9	5.09	77
2002	A4	7/24/2003	MPB	larvae+pupae	live	Red MSMA	0-2	3	3.6	1.3	311.2	6.2	325.3	327.43	99
2002	A5	7/22/2003	MPB	larvae+pupae	live	Red MSMA	0-2	5	2.1	0.4	1.2	0.2	3.9	10.37	38
2002	B1	7/24/2003	MPB	adult	dead	Red MSMA	0-2	3	0.5	1.0	152.9	3.6	162.5	182.26	89
2002	B2	7/17/2003	MPB	adult	dead	Red MSMA	0-2	4	1.7	1.6	244.6	8.4	258.6	354.11	73
2002	B3	7/1/2003	MPB	adult	dead	Red MSMA	0-2	0	0.0	0.6	166.9	3.5	172.4	239.54	72
2002	B4	6/17/2003	MPB	adult	live/dead	Red MSMA	0-2	1	0.2	0.6	91.5	3.3	96.7	140.29	69
2002	B5	6/18/2003	MPB	adult	dead	Red MSMA	0-2	1	0.5	0.6	161.3	3.6	166.5	159.51	104
2002	B6	6/18/2003	MPB	adult	dead	Red MSMA	0-2	0	0.5	0.6	85.1	4.1	92.6	123.54	75
2002	C1	7/17/2003	MPB	larvae+pupae	live	Red MSMA	0-2	1	0.4	0.2	26.1	0.4	27.9	32.80	85

Trt Year	Sample ID	Collection Date	Group	Life Stage	Morbitity	Treatment	Collection Height (m)	Debark Index	As(III)	DMAA	MMAA	As(V)	Total Extract (µg/g)	Total As (µg/g)	Extraction Efficiency (%)
2002	C2	7/22/2003	MPB	larvae+pupae	live	Red MSMA	0-2	3	1.0	1.6	75.9	2.0	80.6	168.82	48
2002	D1	6/30/2003	MPB	adult	dead	Red control	0-2	3						0.02	
2002	D2	6/17/2003	MPB	adult	dead	Red control	0-2	4						1.06	
2002	D3	6/19/2003	MPB	adult	dead	Red control	0-2	1						0.45	
2002	E1	7/17/2003	MPB	larvae+pupae	live	Red control	0-2	4						0.79	
2002	E2	6/30/2003	MPB	larvae+pupae	live	Red control	0-2	3						0.22	
2002	F1	6/19/2003	MPB	larvae+pupae	live	Red control	0-2	1						0.21	
2002	F2	6/17/2003	MPB	larvae+pupae	live	Red control	0-2	4						0.15	
2002	F3	6/30/2003	MPB	larvae+pupae	live	Red control	0-2	3						0.62	
2002	G1	7/17/2003	Ips	adult	live	Red MSMA	0-2	4						19.59	
2002	H1	6/17/2003	MPB	larvae+pupae	live	Red MSMA	0-2	1	0.1	0.1	10.0	0.1	10.5	13.94	75
2003	1	15-Jul-04	MPB	larvae+pupae	live	Red MSMA	0-2	4						13.30	
2003	2	8-Jul-04	MPB	larvae+pupae	live	Red MSMA	0-2	5						3.56	
2003	3	15-Jul-04	MPB	larvae+pupae	live	Red MSMA	0-2	3						20.81	
2003	4	7-Jul-04	MPB	larvae+pupae	live	Red MSMA	0-2	2						68.60	
2003	5	6-Jul-04	MPB	adult	live	Red MSMA	0-2	3						7.09	
2003	6	29-Jun-04	MPB	larvae+pupae	live/dead	Red MSMA	0-2	6						9.05	
2003	7	29-Jun-04	MPB	larvae+pupae	live	Red MSMA	0-2	6						3.03	
2003	8	22-Jun-04	MPB	larvae+pupae	live	Red MSMA	0-2	4						2.97	
2003	9	26-Jul-04	MPB	larvae+pupae	live	Red MSMA	2+	2						14.54	
2003	10	29-Jul-04	MPB	adult	live	Green control	0-2	0						0.72	
2003	11	29-Jul-04	MPB	adult	live	Green control	0-2	0						1.96	
2003	12	22-Jul-04	MPB	IPS	live	Red MSMA	10-12	4						18.48	
2002/2003	13	7-Jul-04	MPB	IPS	live	Red MSMA	0-2	2						5.40	
2003	14	16-Jul-04	MPB	adult	live	Red MSMA	0-2	4						35.41	
2003	15	16-Jul-04	MPB	larvae+pupae	live	Red MSMA	2-4	6						9.97	
2003	16	15-Jul-04	MPB	larvae+pupae	live	Red MSMA	0-2	1						49.82	
2003	17	17/24Jul04	MPB	larvae+pupae	live	Red MSMA	0-2	2						8.76	
2003	18	17/24Jul04	MPB	larvae+pupae	live	Red MSMA	2-4	2						11.94	
2003	19	24-Jul-04	MPB	larvae+pupae	live	Red MSMA	4-6	2						4.97	
2003	20	7-Jul-04	MPB	larvae+pupae	live	Red MSMA	6-8	2						104.34	
2003	21	7-Jul-04	MPB	larvae+pupae	live	Red MSMA	4-6	2						60.47	
2004	1	28-Jun-05	MPB	adult	dead	Red MSMA	0-2	0						127.6	
2004	2	28-Jun-05	MPB	larvae+pupae	live	Red MSMA	0-2	0						36.6	

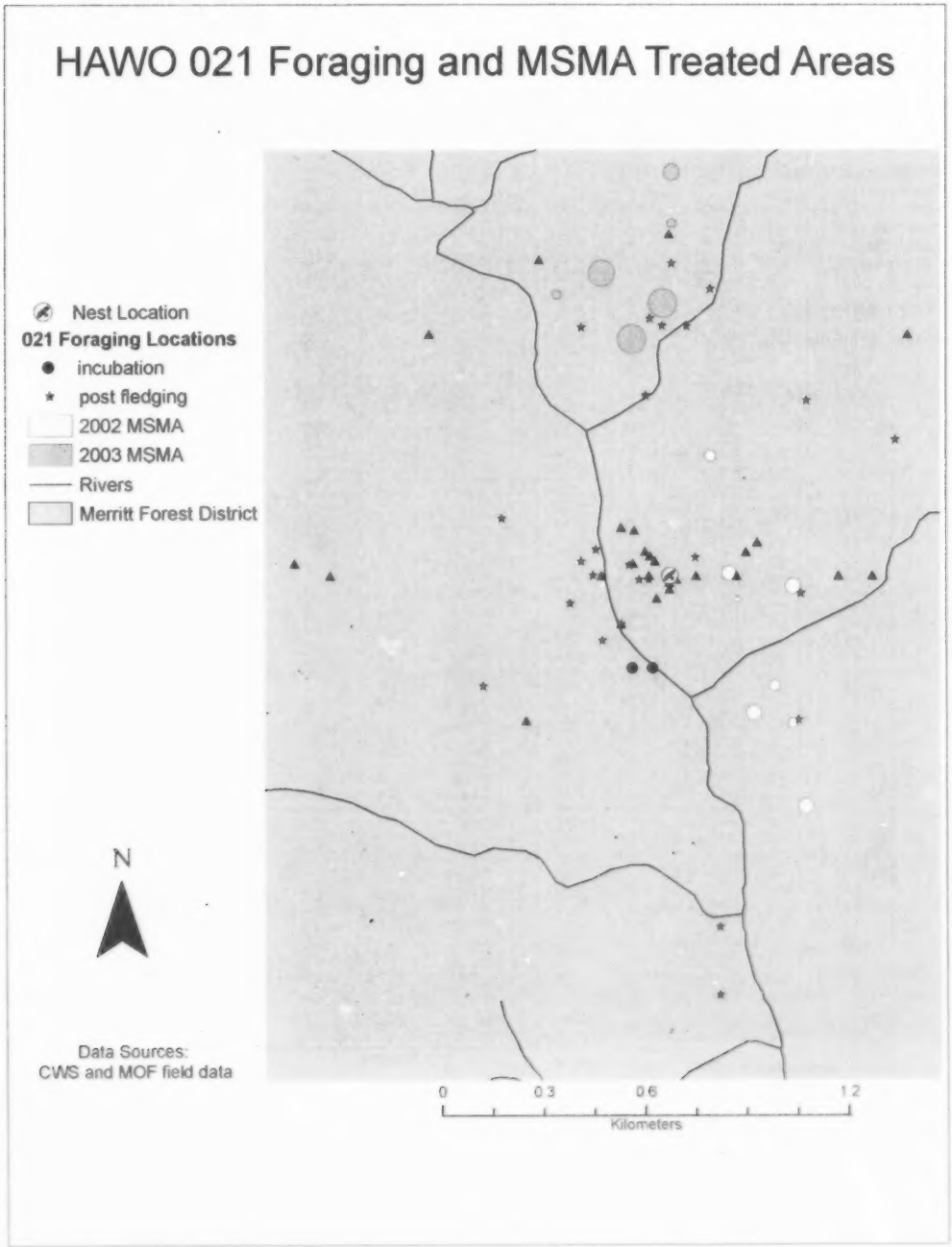
Trt Year	Sample ID	Collection Date	Group	Life Stage	Morbitity	Treatment	Collection Height (m)	Debark Index	As(III)	DMAA	MMAA	As(V)	Total Extract (µg/g)	Total As (µg/g)	Extraction Efficiency (%)
2004	3	28-Jun-05	MPB	larvae+pupae	dead	Red MSMA	0-2	5						447.4	
2004	4	28-Jun-05	MPB	larvae+pupae	live	Red MSMA	0-2	5						4.7	
2004	5	9-Jun-05	MPB	adult	dead	Red MSMA	0-2	0						109.8	
2004	6		MPB	larvae+pupae	live	Red MSMA	0-2	6						40.4	
2004	8	11-Jul-05	MPB	larvae+pupae	dead	Red MSMA	0-2	6						10.6	
2004	11	29-Jun-05	MPB	larvae+pupae	live	Red MSMA	0-2	5						50.9	
2004	12	13-Jul-05	non MPB	adult	live	Red MSMA	0-2	1						37.6	
2004	13	13-Jul-05	MPB	larvae+pupae	live	Red MSMA	0-2	5						18.4	
2004	14		MPB	adult	dead	Red MSMA	0-2	6	0.00	0.00	105.56	4.78	116.34	327.4	35.5
2004	18	12-Jul-05	MPB	larvae+pupae	live	Red MSMA	0-2	3						112.1	
2004	19	12-Jul-05	MPB	larvae+pupae	live	Red MSMA	0-2	1	0.00	1.21	29.01	0.76	31.97	116	27.6
2004	25	7-Jul-05	MPB	larvae+pupae	dead	Red MSMA	0-2	5						233.2	
2004	28	1-Jul-05	MPB	larvae+pupae	live	Green control	0-2	4						0.2	
2004	29	1-Jul-05	MPB	larvae+pupae	live	Green control	0-2	2						0.06	
2004	31	5-Jul-05	MPB	adult	dead	Red MSMA	0-2	1	1.42	2.84	131.52	11.91	0.00	222	0.0
2004	33	11-Jul-05	MPB	larvae+pupae	live	Red MSMA	0-2	6						66.5	
2004	34/35	11-Jul-05	MPB	adult	dead/live	Red MSMA	0-2	5	0.82	0.00	85.05	3.17	94.04	122.7	76.6
2004	38	11-Jul-05	MPB	adult	dead	Red MSMA	0-2	6						208.5	
2004	40	11-Jul-05	MPB	larvae+pupae	live	Red MSMA	0-2	6						1.3	
2004	41	11-Jul-05	non MPB	larvae	live	Red MSMA	0-2	6						5.6	
2004	44	4-Jul-05	MPB	larvae+pupae	live	Red control	0-2	3						0.07	
2004	46	4-Jul-05	MPB	larvae+pupae	live	Red control	0-2	6						0.04	
2004	47	4-Jul-05	MPB	larvae+pupae	live	Red control	0-2	4						0.05	
2004	49	30-Jun-05	non MPB	adult	live	Red MSMA	0-2	1						62.9	
2004	50/51	30-Jun-05	MPB	adult	dead/live	Red MSMA	0-2	1	0.91	0.00	53.48	3.53	58.92	214.7	27.4
2004	52/53	30-Jun-05	MPB	adult	dead	Red MSMA	0-2	1						112.9	
2004	56	30-Jun-05	MPB	larvae+pupae	dead	Red MSMA	0-2	5	0.00	0.00	278.45	5.60	289.05	700.2	41.3

Appendix 2: Details of forest birds trapped and blood/feather sampled in 2004 and 2005 in the Cascades Forest District. All blood samples were analyzed for total arsenic residues. Only selected woodpeckers from 2004 had feathers analyzed for total arsenic. Note: breeding pairs are noted with an asterisk and blood arsenic concentrations were reported as averages in the text and in statistical analysis. HAWO = Hairy woodpecker, MOCH = Mountain chickadee, RBNU = Red breasted nuthatch, RNSA = Red naped sapsucker, TTWO = Three-toed woodpecker.

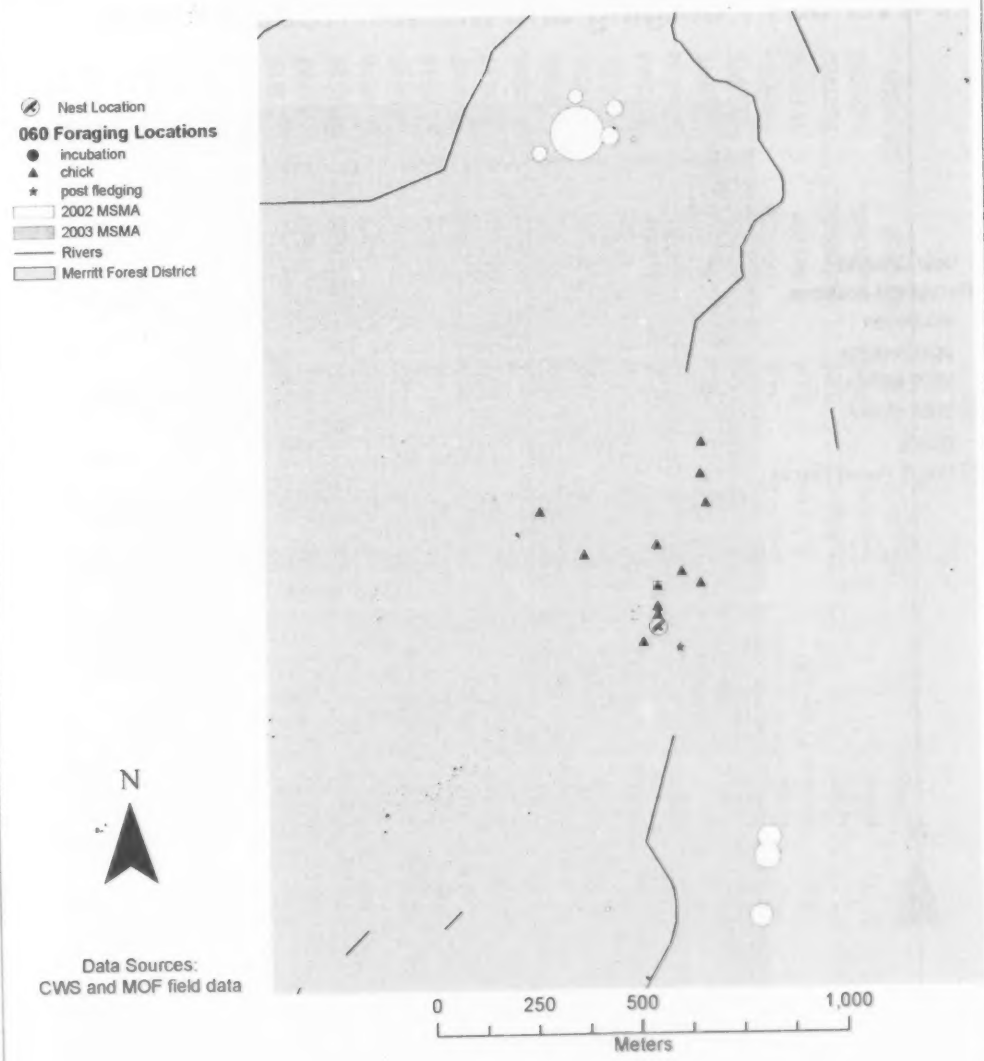
Species	Sex	Radio Frequency	Age	Site	Date	Year	UTM (W-E)	UTM (N-S)	Total As in blood (µg/g)	Total As in feathers (µg/g)
HAWO	male	172.060	ATY	Deadman Lake	29-Apr-04	2004	671926	5514645	0.13	
HAWO	male	172.021	TY	Shrimpton 19km	4-May-04	2004	692254	5517568	0.18	
HAWO	male	172.101	SY	Shrimpton 19km	12-May-04	2004	692254	5517568	0.12	
HAWO*	male	172.120	ATY	Fig Lake	14-May-04	2004	648246	5525714	0.11	
HAWO*	female		ASY	Fig Lake	1-Jun-04	2004	648246	5525714	0.21	
HAWO	female	172.260	SY	Hornet	26-May-04	2004	671415	5513316	0.21	1.13
HAWO	female		TY	Batstone Lk	28-May-04	2004	672072	5516404	0.15	3.95
HAWO	female		TY	Ketchan	1-Jun-04	2004	676570	5510574	0.13	0.46
HAWO	female	172.152	ATY	Elusive 17km	31-May-04	2004	691368	5518963	0.27	0.19
HAWO*	male		SY	Englishman Lake	8-Jun-04	2004	663644	5535813	0.36	2.78
HAWO*	female	172.172	SY	Englishman Lake	7-Jun-04	2004	663644	5535813	0.34	0.20
HAWO	male		SY	Englishman Lake	8-Jun-04	2004	664015	5535341	0.53	0.50
HAWO*	male		SY/TY	Thynne	20-May-05	2005	0654940	5517519	0.07	
HAWO*	female	172.210	ATY	Thynne	25-May-05	2005	0654940	5517519	0.12	
HAWO	male	172.291	SY	Goose	26-May-05	2005	0663553	5513526	0.05	
HAWO	male	172.339	ATY	Cattleguard	31-May-05	2005	0661452	5522270	0.30	
HAWO	male	172.409	SY	Coley clearcut	31-May-05	2005	0660438	5520564	0.14	
HAWO	male	172.440	ATY	Thalia	1-Jun-05	2005	0664511	5514795	0.12	
HAWO	male		ASY	Swa11	15-Jun-05	2005	0668100	5578000	0.40	
MOCH	unknown		AHY	Goose A	3-Jun-05	2005	663489	5513379	1.05	
MOCH	unknown		AHY	Goose B	3-Jun-05	2005	663524	5513283	0.90	
MOCH	unknown		AHY	Coley Box	10-Jun-05	2005	660241	5521363	0.28	
MOCH	unknown		AHY	Helmer	13-Jun-05	2005	667871	5573396	2.20	
MOCH	unknown		AHY	Thalia Box	18-Jun-05	2005	664010	5514848	0.52	
MOCH	unknown		AHY	Goose B	18-Jun-05	2005	663524	5513283	0.19	

Species	Sex	Radio Frequency	Age	Site	Date	Year	UTM (W-E)	UTM (N-S)	Total As in blood (µg/g)	Total As in feathers (µg/g)
MOCH	unknown		AHY	Youngs 1.5km	18-Jun-05	2005	666670	5513941	0.26	
MOCH	unknown		AHY	Davis 2km	19-Jun-05	2005	664399	5522905	0.22	
MOCH	unknown		AHY	Crunch	20-Jun-05	2005	663305	5521222	0.12	
MOCH	unknown		AHY	AngBang	21-Jun-05	2005	661077	5522076	0.08	
MOCH*	unknown		AHY	Davis BR	23-Jun-05	2005	663942	5523461	0.03	
MOCH*	unknown		AHY	Davis BR	23-Jun-05	2005	663942	5523461	0.11	
MOCH	unknown		AHY	Crunch	23-Jun-05	2005	663305	5521222	0.29	
MOCH	unknown		AHY	Coley BR	23-Jun-05	2005	659975	5520819	0.02	
MOCH	unknown		AHY	Davis 4km	24-Jun-05	2005	663538	5524723	0.14	
MOCH*	unknown		AHY	Davis Aspen	24-Jun-05	2005	663608	5524516	0.17	
MOCH*	unknown		AHY	Davis Aspen	24-Jun-05	2005	663608	5524516	0.29	
MOCH*	unknown		AHY	Coley BR high	24-Jun-05	2005	659975	5520849	0.08	
MOCH*	unknown		AHY	Coley BR high	24-Jun-05	2005	659975	5520849	0.11	
RBNU*	male		ASY	Johnny Lake	22-Jun-05	2005	0663296	5513447	0.06	
RBNU*	female		SY	Johnny Lake	22-Jun-05	2005	0663296	5513447	0.07	
RNSA*	female		AHY	Elusive 17km-S11	30-Jun-04	2004	691368	5518963	0.04	0.13
RNSA*	male		AHY	Elusive 17km-S11	30-Jun-04	2004	691368	5518963	0.05	0.32
RNSA	female		SY/TY	Davis	24-Jun-05	2005	0664067	5523495	0.05	
RNSA	female		SY/TY	towards Lodwick	24-Jun-05	2005	0664202	5514194	0.05	
RNSA	unknown		SY/TY	Davis	24-Jun-05	2005	0664237	5523548	0.16	
RNSA	female		AHY	Davis rd	16-Jun-05	2005	0662636	5524494	0.16	
RNSA*	male		SY	Davis #3	16-Jun-05	2005	0663635	5524913	0.55	
RNSA*	female		AHY	Davis #3	2-Jun-05	2005	0663635	5524913	3.73	
RNSA	male		AHY	Bang-Crunch clearcut	8-Jun-05	2005	0664237	5523548	0.65	
TTWO	male	172.238	SY	Elusive 17km-S11	8-Jul-04	2004	691226	5519350	0.34	1.39
TTWO*	male		SY/TY	Coley	25-May-05	2005	0660008	5521282	0.10	
TTWO*	female	172.501	SY/TY	Coley	5-May-05	2005	0660008	5521282	0.03	
TTWO	female	172.464	AHY	Coley #2	15-Jun-05	2005	0659950	5520806	0.08	

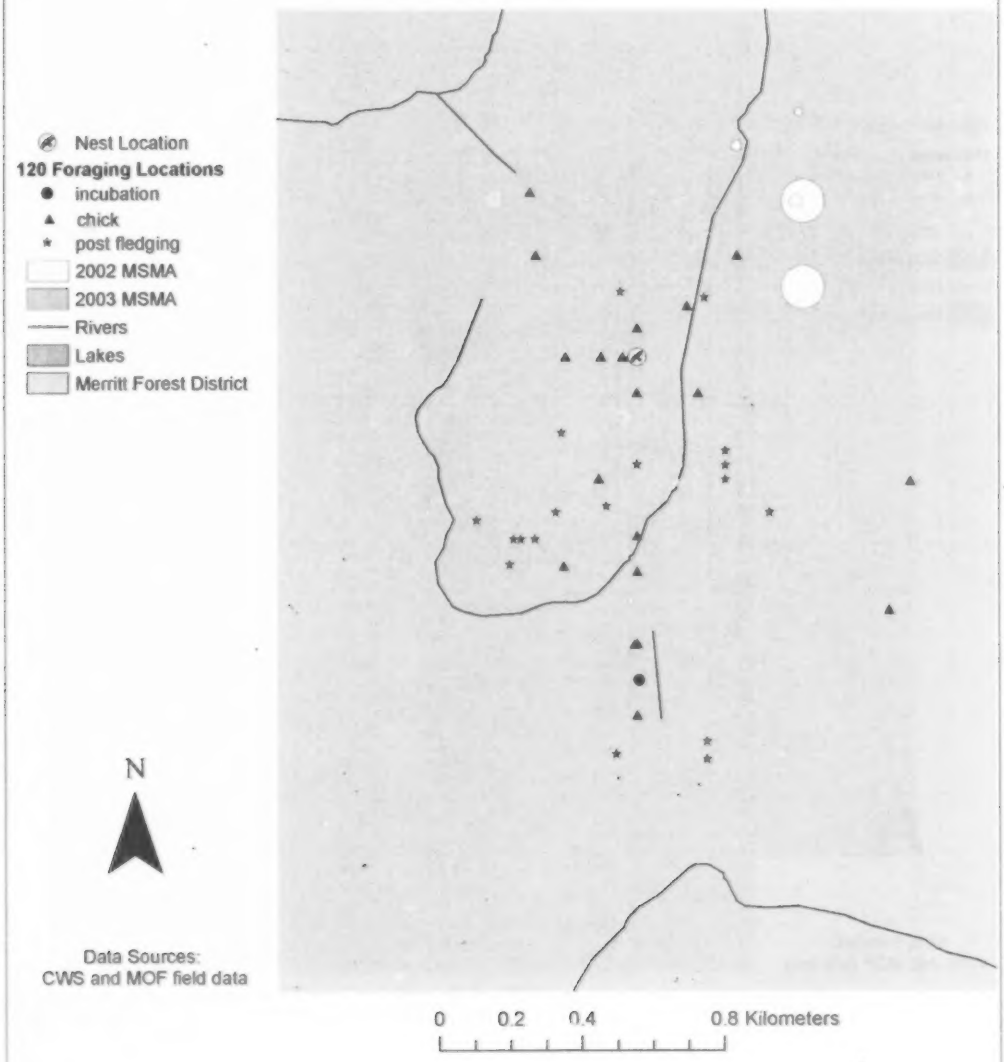
Appendix 3a-n: Maps of foraging locations of individual radio tagged woodpeckers (Hairy and Three toed woodpeckers) observed during radio telemetry tracking and recent MSMA treatment areas (2002, 2003, 2004). Woodpecker locations are grouped by breeding phase (incubation, chick rearing, and post fledging/post breeding.



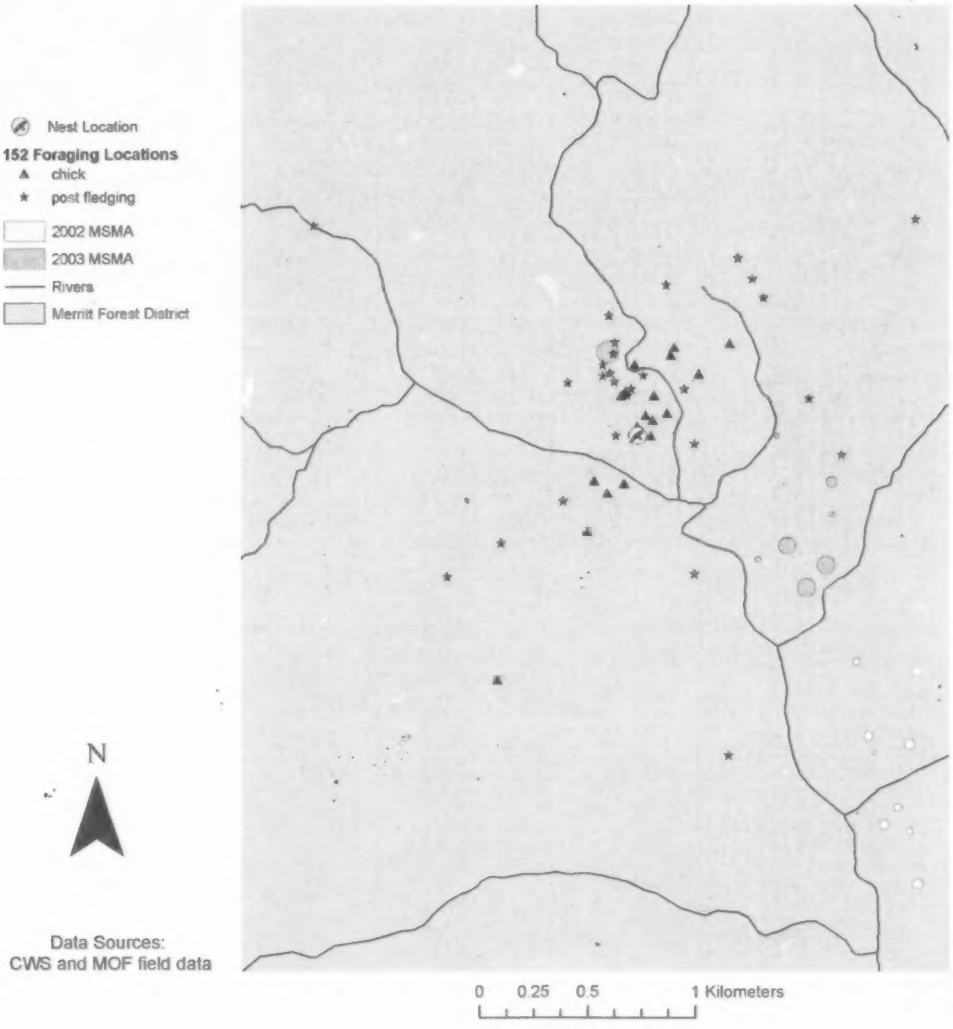
HAWO 060 Foraging and MSMA Treated Areas



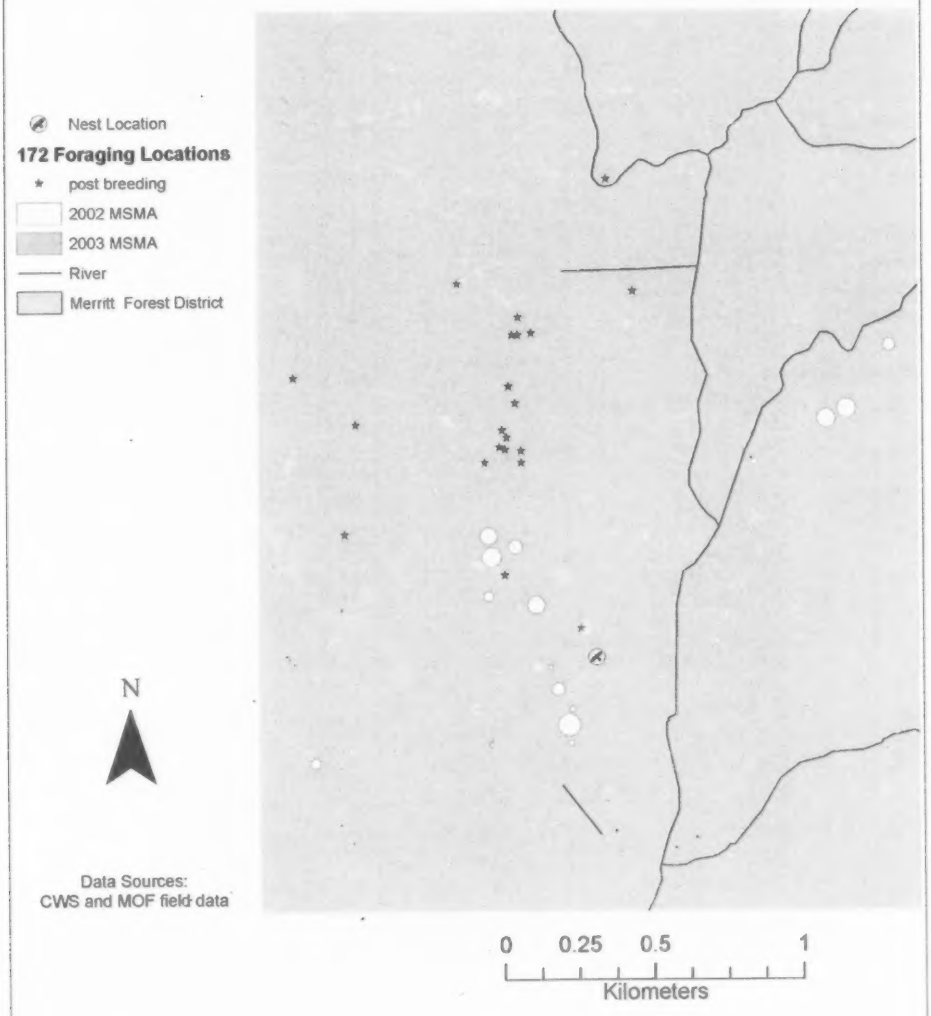
HAWO 120 Foraging and MSMA Treated Areas



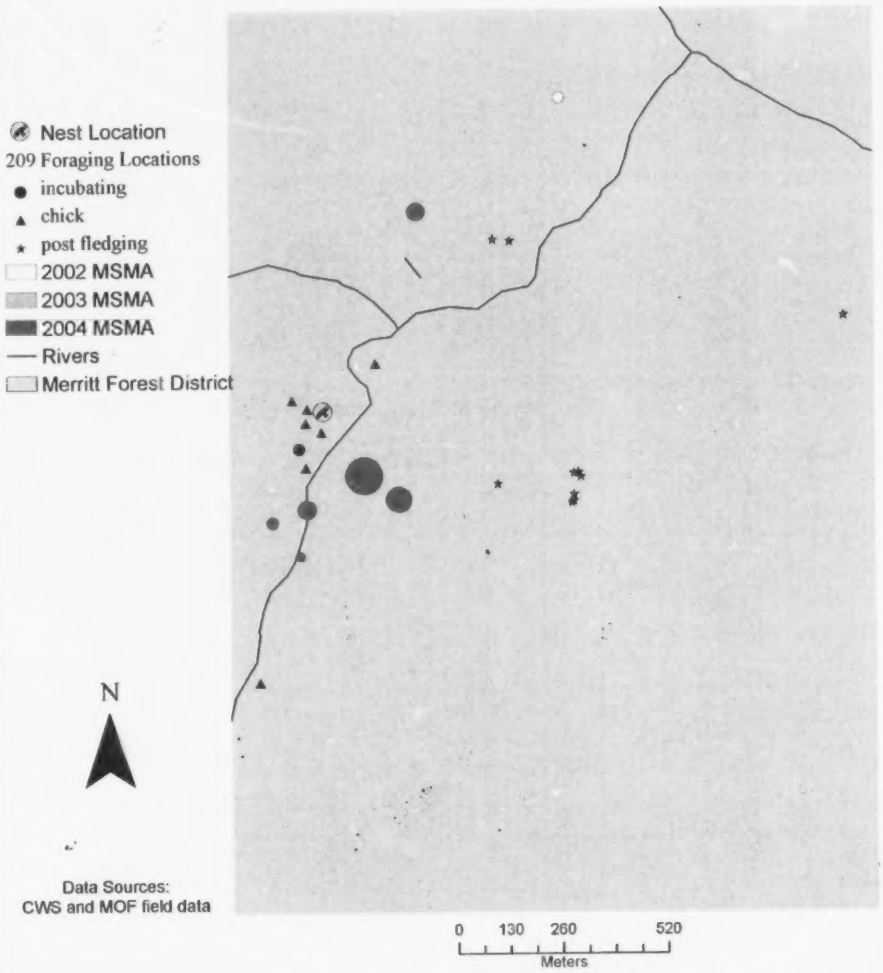
HAWO 152 Foraging and MSMA Treated Areas



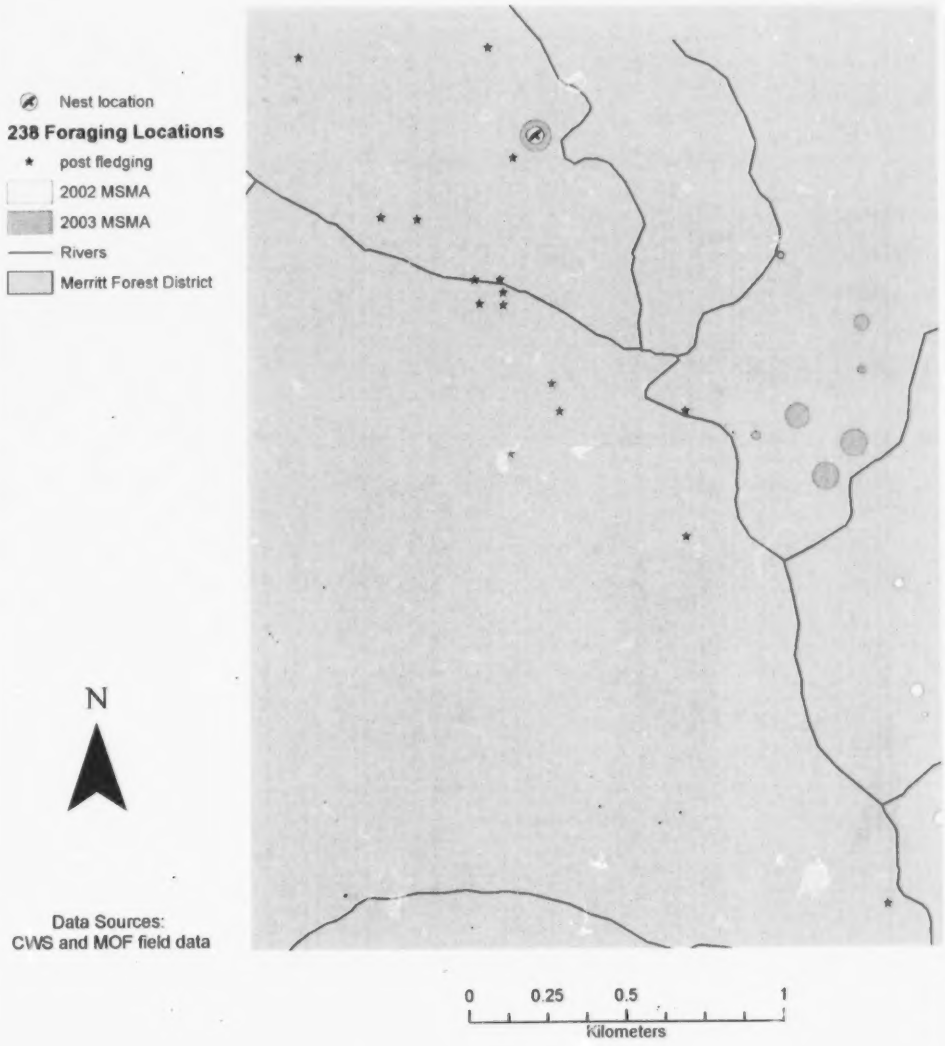
HAWO 172 Foraging and MSMA Treated Areas



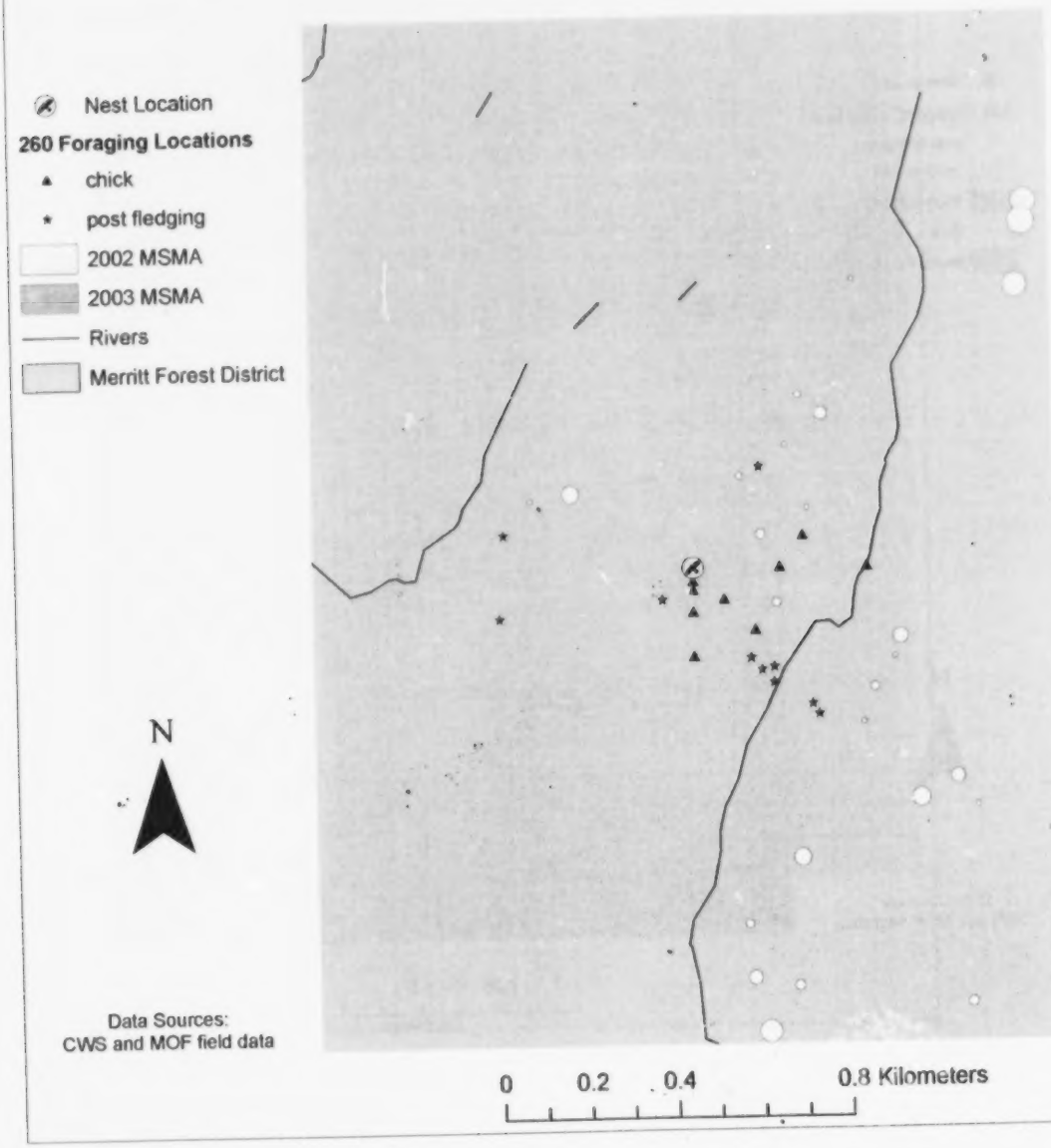
HAWO 209 Foraging and MSMA Treated Areas



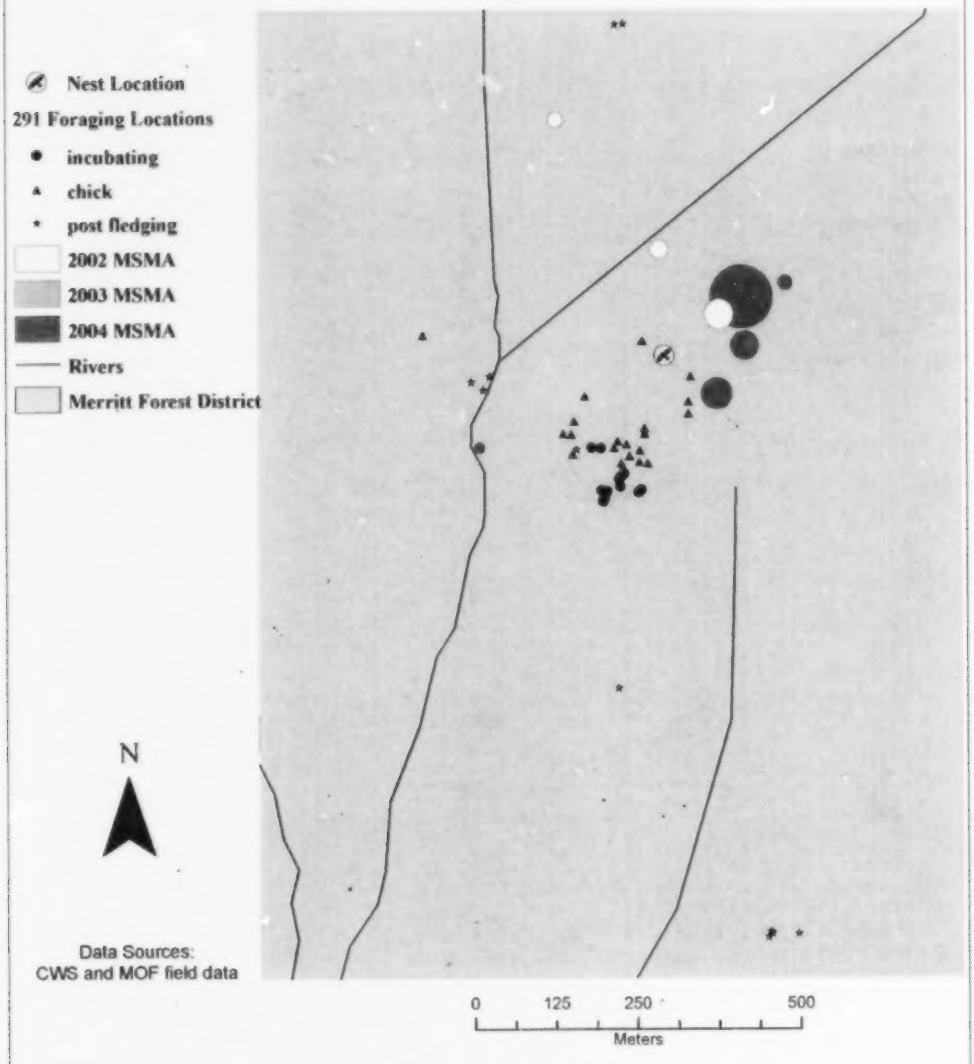
TTWO 238 Foraging and MSMA Treated Areas



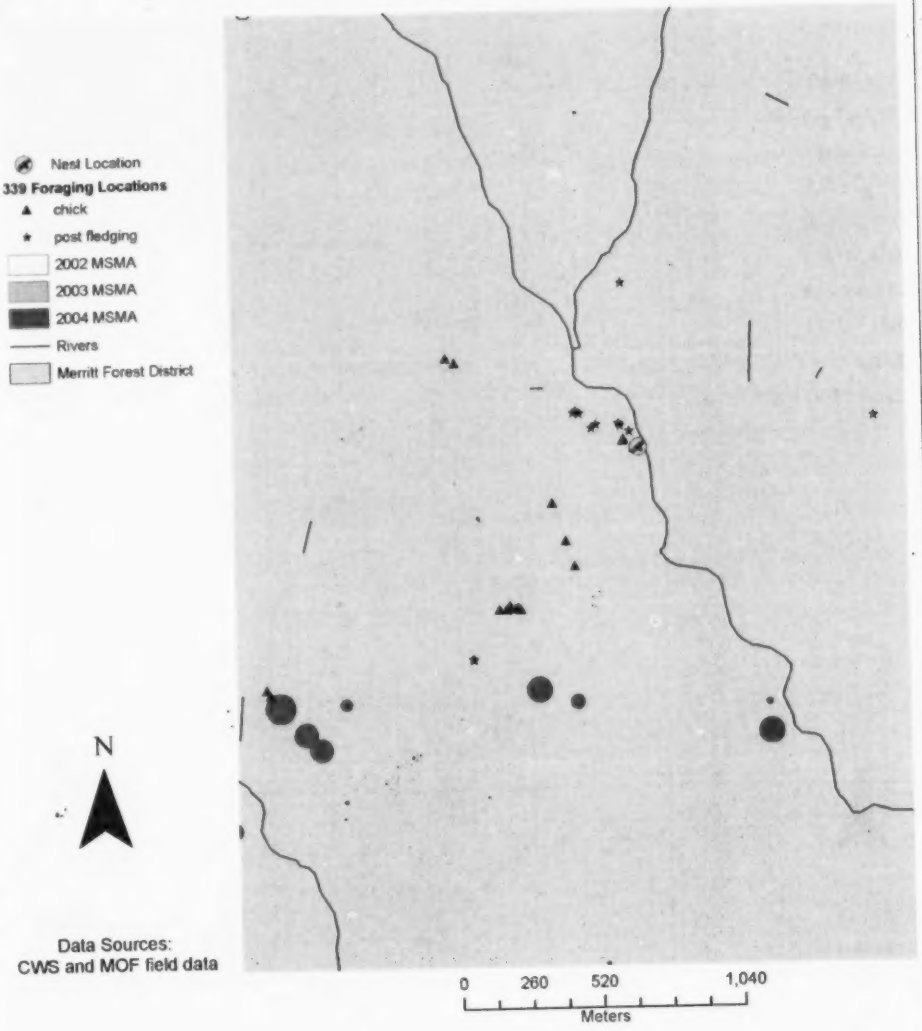
HAWO 260 Foraging and MSMA Treated Areas



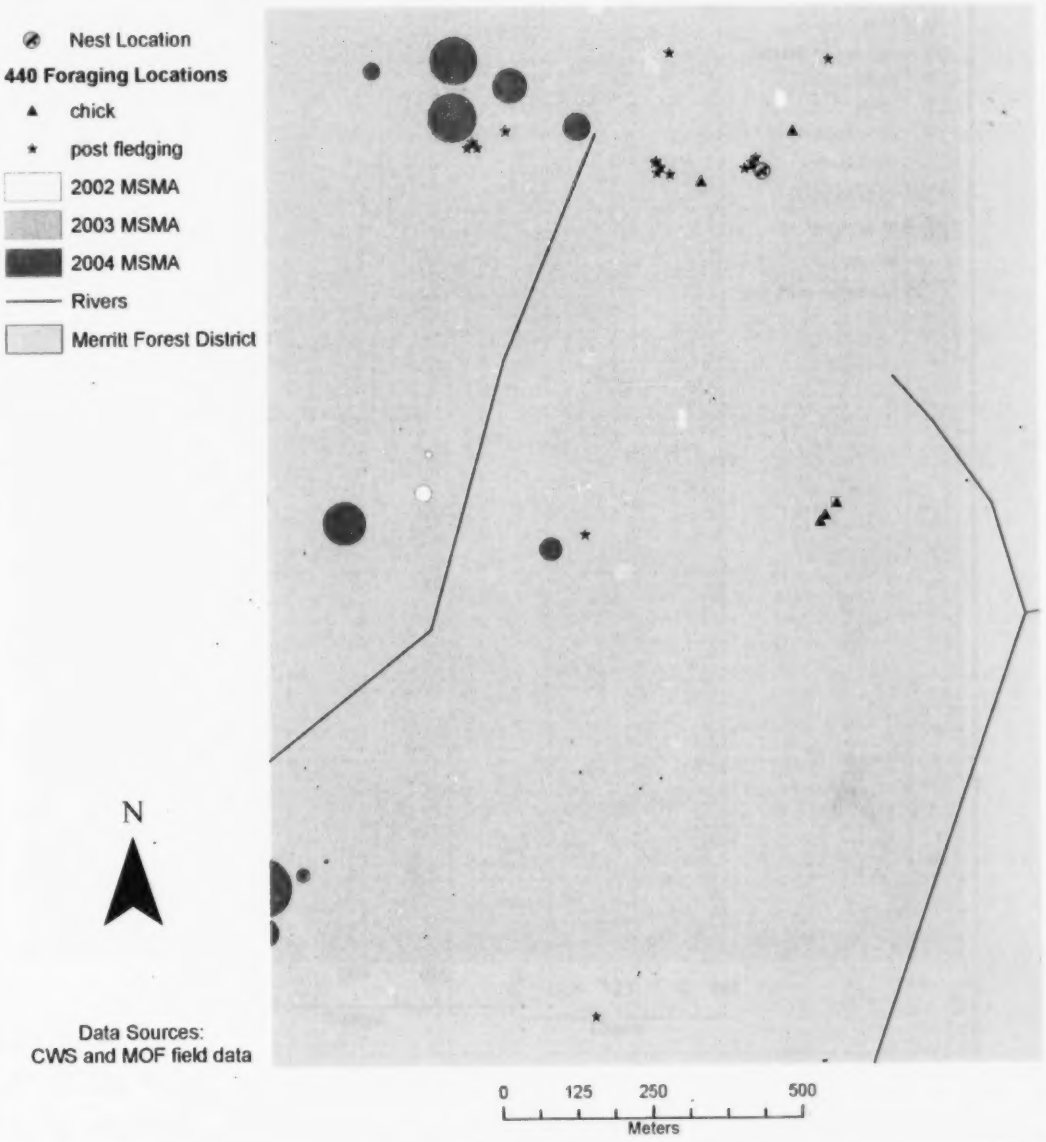
HAWO 291 Foraging and MSMA Treated Areas



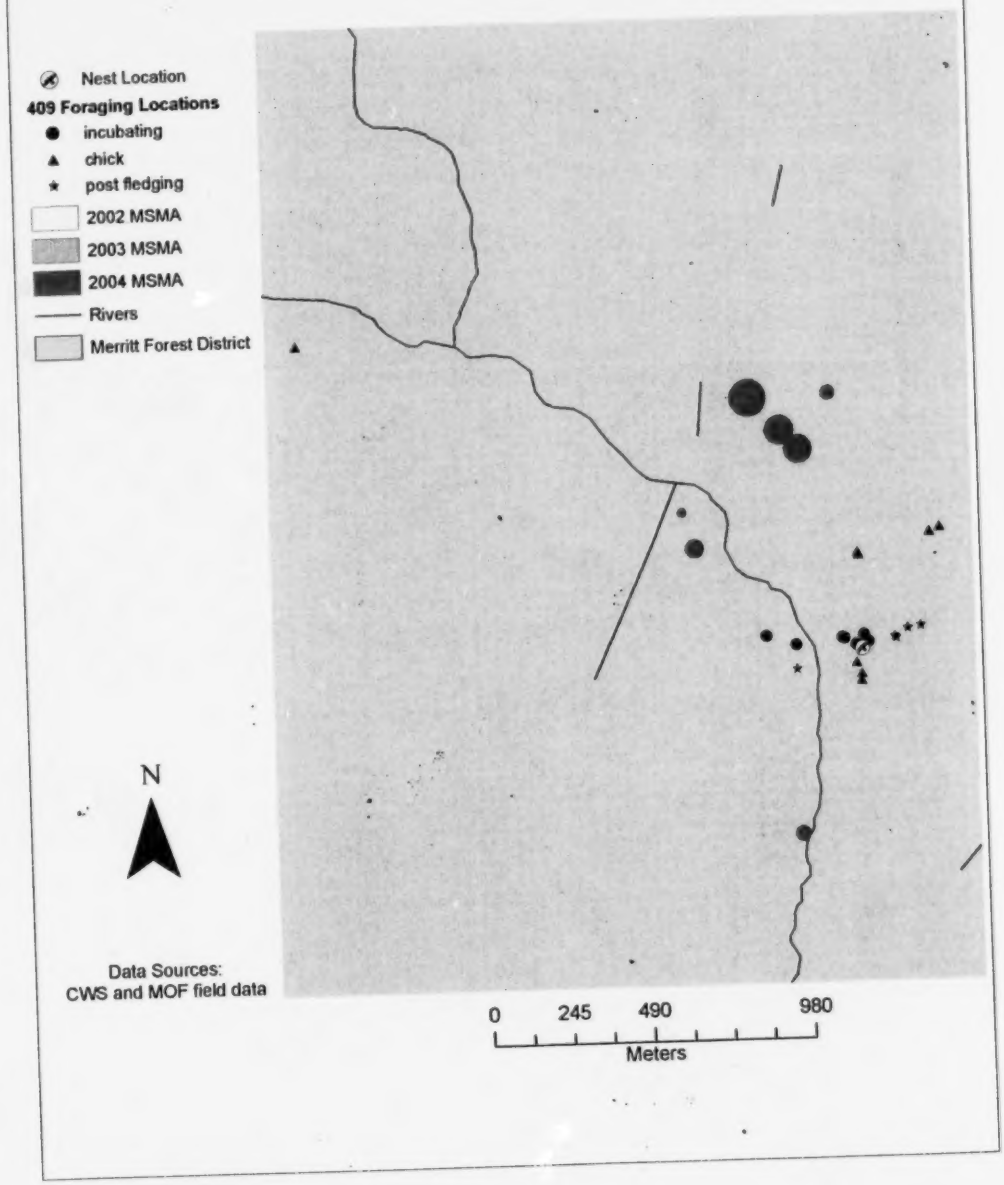
HAWO 339 Foraging and MSMA Treated Areas



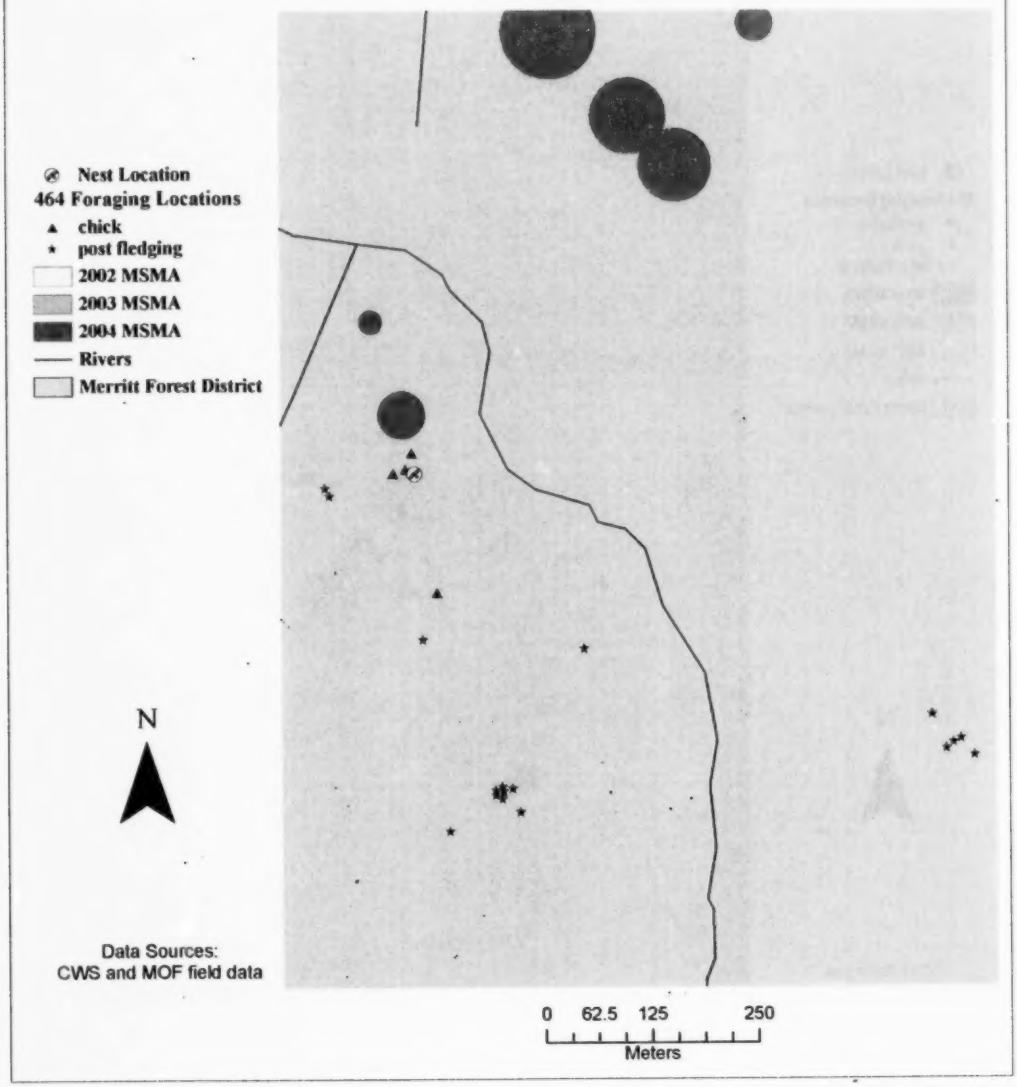
HAWO 440 Foraging and MSMA Treated Areas



HAWO 409 Foraging and MSMA Treated Areas



TTWO 464 Foraging and MSMA Treated Areas



TTWO 501 Foraging and MSMA Treated Areas